Int. J. Cancer: **123,** 2727–2734 (2008)

MINI REVIEW

How to minimize formation and growth of tumours: Potential benefits of decapod crustaceans for cancer research

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Tumours have only rarely been observed in the decapod crustaceans, a large animal group of more than 10,000 species that includes the commercially important and well investigated shrimp, lobsters, crayfish and crabs. Analysis of the literature and information from cancer and diseases data bases revealed a total of 15 incidences, some of them being questionable. Even in the long-lived species, which can reach life spans of almost 100 years, neoplasias are virtually unknown. The data published so far suggest that the strikingly different frequencies of carcinogenesis between decapods and other well investigated animal groups like mammals, fish, insects and molluscs is based on differences of the metabolic pathways for carcinogens, the immune systems, and the regulation of stem cells. Therefore, representatives of the Decapoda may serve as useful models to study how organisms can successfully prevent or control spontaneously and environmentally induced cell proliferation. A particularly promising candidate for in-depth investigation of these topics is the marbled crayfish, a rather new clonal lineage that is presently being introduced as a laboratory model in development and epigenetics.

Key words: neoplasia; Decapoda; Crustacea; stem cells; detoxification of carcinogens; age-related cancer; immune system; epigenetics; marbled crayfish

The decapod crustaceans are conspicuous and abundant marine and freshwater invertebrates comprising more than 10,000 species. They are a phylogenetically old taxon dating back to the late Devonian (~360 million years).[1] The Decapoda provide keystone species in numerous aquatic habitats and are intensely exploited for human consumption. The total global production of shrimp, lobsters, crabs, sea-spiders, spiny-rock lobsters and freshwater crayfish by fisheries and aquaculture amounted to 9.3 million tons in 2005, corresponding to a value of more than US$20 billion.[2] Due to their ecological and economical importance and their traditional use as experimental animals in various biological disciplines,[3] the Decapoda are amongst the best investigated invertebrates.

Despite the good data base on the biology of the decapod crustaceans there are only very few reports on neoplasias. This scarcity of reports apparently reflects a low incidence of tumours in the Decapoda compared to other well investigated animal groups rather than low research efforts in this direction.[4–10] In the framework of environmental monitoring, aquaculture biosecurity programmes and seafood quality controls wild and cultured populations of decapod species are regularly investigated for their health status and diseases.[11–13] As a result of these surveys and additional routine checks of aquarium and laboratory populations almost 200 different diseases have been described in the decapod crustaceans,[14–20] which is in sharp contrast to the small number of tumour incidences published.

This review starts with a summary of the literature on spontaneous and environmentally induced neoplasias in the decapod crustaceans. It then addresses the peculiarities of the detoxification pathways of carcinogens, the potential role of the immune system in prevention of tumour formation and the specificities of stem cells in the Decapoda. The article ends with a discussion on the cancer research potential of the Decapoda, propagating the use of a new crayfish laboratory model for future investigations in this direction.

Neoplasias in the Decapoda in comparison to insects, molluscs and fish

There are only 15 reports on neoplasias in the decapod crustaceans (Table I) and some of them are of dubious validity since they are only briefly mentioned in articles primarily concerned with other topics. Scharrer and Lochhead[4] and Sparks and Lightner[21] summarized the older reports, which cover a total of 6 incidences in the spider prawn, *Nematopalaemon tenuipes*, the American lobster, *Homarus americanus*, the hermit crab, *Pagurus longicarpus*, and the common shore crab, *Carcinus maenas* (Table I). These incidences include tumour-like growths on the body side, on a regenerating chela, in a stomach wall, in a parasitized abdomen and in a parasitized gill chamber. Most of these older reports are questionable because the tumour-like growths have not been subjected to histopathological examination. Also questionable is the description of a haemic neoplasm in the tropical field crab, *Paratelphusa hydrodromous*, after exposure to sublethal concentrations of cadmium chloride.[22]

The few remaining neoplasias have been described by experienced crustacean pathologists. Some of these tumours are apparently of spontaneous origin because they were found in single individuals only. Sparks and Lightner[21] detected a papilliform tumour-like growth of 9 × 8 × 8 mm on the ventrolateral aspect of the pleon (abdomen) of an adult brown shrimp, *Farfantepenaeus aztecus*, from an aquaculture pond in Texas and diagnosed it as a benign neoplasm. The tumour originated from the epidermis and the subepidermal tissue and consisted of grossly hypertrophied and normal tissue elements. Three further discrete neoplasia-like growths are listed by the Registry of Tumors in Lower Animals (RTLA; http://www.pathology-registry.org/search/SumOutTaxonomy.asp), namely an epidermal papilloma in a cultured adult yellowleg shrimp, *Farfantepenaeus californiensis*, from Mexico, another epidermal papilloma in a pond-reared juvenile Pacific white shrimp, *Litopenaeus vannamei*, from Arizona, and a carcinoma in the antennal gland of an adult blue king crab, *Paralithodes platypus*, from Alaska (Table I).

Aside of these spontaneous neoplasias there are 2 incidences that are perhaps related to environmental carcinogens. Lightner and Hedrick[23] detected an embryonic carcinoma in about 20% of the embryos of 3 brooding grass shrimp, *Palaemon orientis*, collected from a penaeid shrimp culture facility in Taiwan. The lesions were characterized by disorganization of embryonic tissues and high amounts of undifferentiated pleomorphic cells with hypertrophied nuclei and prominent nucleoli. Tripolar and bizzare multipolar mitotic figures were frequently found. The authors speculated that these carcinomas may have been caused by a virus infection or an unidentified environmental carcinogen. Overstreet and Van Devender[11] detected tumour-like growths of the striated-musculature in the pleon of 33 postlarvae of the brown shrimp, *Farfantepenaeus aztecus*, and the white shrimp, *Litopenaeus*

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Received 1 August 2008; Accepted after revision 13 August 2008
DOI 10.1002/ijc.23947
Published online 29 September 2008 in Wiley InterScience (www.interscience.wiley.com).

TABLE I – NEOPLASIA-LIKE LESIONS IN DECAPOD CRUSTACEANS

Species	Lesion	Locality	Reference/RTLA no.
Nematopalaemon tenuipes[1]	Tumour in parasitized branchial chamber	?	Sparks and Lightner[21]
Homarus americanus[1]	Large tumour in stomach wall	?	Scharrer and Lochhead[4]
Homarus americanus[1]	Large growth on side	Massachusetts, wild	Sparks and Lightner[21]
Homarus americanus[1]	Growth on side	?	Sparks and Lightner[21]
Paratelphusa hydrodromous[1]	Haemic neoplasia after cadmium chloride treatment	laboratory	Victor[22]
Pagurus longicarpus[1]	Abnormal growth on regenerating chela after exposure to *p*-thiocresol	laboratory	Scharrer and Lochhead[4]
Carcinus maenas[1]	Tumour-like tissue in parasitized abdomen	?	Sparks and Lightner[21]
Palaemon orientis	Embryonal carcinoma	Taiwan, culture	Lightner and Hedrick[23]
Farfantepenaeus aztecus	Papilliform tumour-like growth on pleon	Texas, culture	Sparks and Lightner[21]; RTLA 725
Farfantepenaeus aztecus and Litopenaeus setiferus	Hamartoma of musculature in pleon	Mississippi, wild, polluted area	Overstreet and Van Devender[11]; RTLA 1663
Litopenaeus vannamei	Lymphoma-like neoplasia arising from haematopoietic tissue	Hawaii, culture	Lightner and Brock[25]; RTLA 3749
Litopenaeus vannamei	Papilloma of epidermis	Arizona, culture	RTLA 7622
Farfantepenaeus californiensis	Papilloma of epidermis	Mexico, culture	RTLA 1710
Paralithodes camtschaticus	Carcinoma-like neoplasm in hindgut	Alaska, wild	Sparks and Morado[24]
Paralithodes platypus	Carcinoma in antennal gland	Alaska, wild	RTLA 3466

[1]Dubious validity: either no illustration, no histopathological examination, or misinterpretation.–RTLA no, accession number of the Registry of Tumors in Lower Animals (RTLA): http://www.pathology-registry.org/search/SumOutTaxonomy.asp.

setiferus, in the course of a semi-monthly monitoring programme over more than 5 years in the Mississippi estuary. The growths protruded through the ventral portion of the abdomen and were classified as hamartomas. The afflicted individuals represented only a small proportion of the shrimp examined (0.56% of 2,320 white shrimp, 0.076% of 26,238 brown shrimp and none of 4,573 pink shrimp, *Farfantepenaeus duorarum*) but nearly all such individuals were collected from the most heavily polluted site. Therefore, the authors suggested an unidentified pollutant as the cause of the abnormality.

Two of the 15 neoplasias described for the Decapoda were clearly invasive. Sparks and Morado[24] reported on a carcinoma-like neoplasm in an adult red king crab, *Paralithodes camtschaticus*, which was detected in the course of a long-term survey of diseases of Alaskan king crabs. The tumour presented as a white lump of 20 × 12 × 12 mm that was attached to the anterior-most hindgut. It was massively invasive in some areas and included large pleomorphic epithelioid cells with markedly hypertrophied nuclei, sparse chromatin and prominent nucleoli (Fig. 1*a*). Lightner and Brock[25] described a lymphoma-like neoplasm in an adult female of the Pacific white shrimp, *Litopenaeus vannamei*, that was found in Hawaii during routine examination of cultured shrimp for diseases. The tumour nodules contained numerous anaplastic and hypertrophied lymphoid cells, giant cells with either one large nucleus or several small nuclei (Fig. 1*b*), and polypolar mitotic figures (Fig. 1*c*). This neoplasm originated from haematopoietic tissue and caused metastatic foci in several organs. Further histopathological features indicated an infection of these shrimp with the penaeid shrimp virus IHHN and suggest a relationship between the disease and the neoplasia.

The low natural incidence of tumours in the Decapoda is confirmed by the complete lack of information on growth abnormalities in the numerous articles and books on the histology and cytology of decapod crustaceans.[3,26,27] Moreover, in the thousands of shrimp, prawns and freshwater crayfish, which I have sectioned or inspected during the last 20 years in the course of my research on the structural dynamics of tissues and cells of decapods due to endogenous and environmental factors, I have found numerous viruses, bacteria, fungi, protozoan and metazoan parasites, and lesions caused by food ingredients, heavy metals and toxic chemicals but no tumours.[3,17,18,28–32]

The low frequency of neoplasias in the decapod crustaceans is in sharp contrast to the rather frequent occurrence of tumours in other well investigated invertebrate groups like the bivalves[6–9,33] and insects[4,5,34] or vertebrates like fish,[6,8,35,36] rodents[37,38] and man.[39] Hundreds if not thousands of papers are available on neoplasias in bivalves, insects or fish, which are, like the decapod crustaceans, not in the main focus of cancer research. Further information and literature on neoplasias in these taxa are found in the RTLA database. The tumour incidences reported for rodents, cats and dogs, the main animal models for cancer research are innumerable.

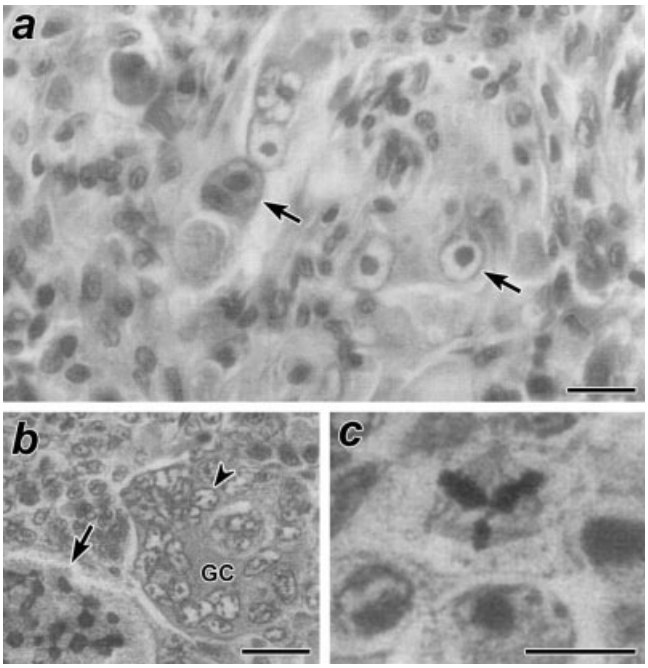

FIGURE 1 – Examples of neoplasias in decapod crustaceans. (*a*) Tumour in the hindgut of *Paralithodes camtschaticus* showing neoplastic cells with large nuclei and prominent nucleoli (arrows). Light microscopy, H&E staining. Bar: 20 µm. (Reproduced from J Invertebr Pathol, 50, Sparks and Morado, 45–52. Copyright Elsevier (1987) with permission of the Publisher.[24]) (*b–c*) Lymphoma-like neoplasm arising from haematopoietic tissue in *Litopenaeus vannamei*. The tumour includes anaplastic cells with nuclei of highly variable size (*b*), giant cells (GC) with numerous small nuclei (*b*, arrowhead) and aberrant polypolar mitotic figures (*c*). Arrow in (*b*) denotes mitotic giant cell. Light microscopy, H&E staining. Bars: 20 µm (*b*) and 10 µm (*c*). (Reproduced from J Invertebr Pathol, 49, Lightner and Brock, 188–193. Copyright Elsevier (1987) with permission of the Publisher.[25])

Effects of environmental carcinogens on decapod crustaceans and peculiarities of their detoxification pathways

Carcinogenic environmental chemicals and carcinogenic food ingredients are major inducers of tumours in animals and humans.[40,41] In polluted aquatic ecosystems carcinogens like polycyclic aromatic hydrocarbons (PAH) or polychlorinated biphenyls (PCB) are often present in the water column and the food chain but are particularly concentrated in the sediment. Therefore, benthic animals like bottom feeding fish, molluscs and decapod crustaceans are at the highest risk to be contaminated with such carcinogens and to develop neoplastic diseases. Consequently, there are numerous papers on tumours in teleost fishes from polluted areas with prevalences up to 50% and even 100% in exceptional cases.[8,35] The incidences reported include practically all tumour types known from mammals. Less but still many cases of neoplasias are known for molluscs from polluted areas, often with prevalences higher than 30%.[42,43] The most frequently found neoplasias in the bivalves and gastropods were disseminated neoplasias and germinomas but there were also some other types of tumours.

Interestingly, decapod crustaceans caught from the same neoplasia-inducing environments or similarly polluted areas have not developed tumours or pre-neoplastic changes.[44] The only abnormal growth in a decapod that is likely to be linked to environmental pollutants is the hamartoma found by Overstreet and Van Devender[11] in postlarvae of brown and white shrimp from the estuary of the Mississippi. Laboratory experiments have principally corroborated the differences in sensitivity to carcinogenic chemicals between fish, molluscs and decapod crustaceans as found in the wild. By exposure to water-borne carcinogens a broad variety of tumours was induced in fish but a less broad spectrum in bivalves. In decapod species such experiments revealed no tumours although the experimental animals had been exposed to the same concentrations of the test carcinogens that had elicited tumours in fish.[6,36,45–47]

James *et al*. searched for the reasons of the high resistance of decapod crustaceans to carcinogenic chemicals using the American lobster, *Homarus americanus*, as the experimental animal and benzo[*a*]pyrene (BaP) as the model carcinogen.[44,48,49] BaP is a PAH and is widespread in aquatic ecosystems. It is one of the best investigated carcinogens, and thus, it is often used as a model substance in laboratory experiments on carcinogenesis.[50] In mammals, this substance produced tumours at multiple sites in all species tested. BaP is a pro-carcinogen that is metabolized by cytochrome P-450 to reactive intermediates, which can covalently bind to DNA. These DNA adducts can cause mutations in critical genes and thus initiate carcinogenesis.[51] Cytochrome P-450 has similar roles in activation of PAH carcinogens in mammals, fish and invertebrates, but usually displays lower rates of activity in the invertebrates.[52]

James *et al*. found that after both intrapericardial injection and oral administration of radiolabelled BaP most of the retained chemical was stored as the parent compound for long periods of time (half life: ~30–60 days) in the lipid-rich hepatopancreas and to a lesser degree in the musculature.[44] The hepatopancreas is the main metabolic organ of the Decapoda and is in many aspects comparable with the vertebrate liver.[3] The BaP was only slowly biotransformed to phase-1 metabolites such as BaP-7,8-dihydrodiol, BaP-1,6-, 3,6- and 6,12-diones and hydroxy BaPs. These metabolites were subsequently converted into phase-2 metabolites such as glucoside, sulfate and glutathione conjugates and rather rapidly excreted, as demonstrated for 9-hydroxy-BaP (half-life: ~50–100 hr).[44,48] The phase-1 metabolites formed adducts with the DNA but only to a very low degree.[44] These biotransformation and pharmacokinetics studies indicate that the major reason for the resistance of the American lobster to PAH induced cancers is the very slow phase-1 metabolism to reactive metabolites and the rapid clearance of these intermediates *via* conversion to phase-2 metabolites and excretion. This specific metabolic pathway seems to drastically reduce the probability of DNA adduct formation.

A different picture was found in the spiny lobster, *Panulirus argus*, (Decapoda, Achelata) that, within the Decapoda, is distantly related to the American lobster (Decapoda, Homarida). In vitro studies showed that hepatopancreatic cytochrome P-450 metabolized BaP rapidly to phase-1 products, which then covalently bound to exogeneous DNA.[49] In vivo experiments corroborated the rapid metabolization of the parent compound. The BaP intermediates formed adducts with the DNA but these were efficiently eliminated from the tissue and not as persistent as in BaP treated fish or rat.[49,53]

A high capacity of decapod crustaceans to detoxify carcinogenic substances was not only found for PAH but also for polychlorinated biphenyls, although these detoxification pathways are not yet known in detail.[54]

Elimination of neoplastic cells by the immune system

There is overwhelming evidence that the immune system of vertebrates can not only recognize and eliminate primary developing tumours (cancer immunosurveillance) but can also promote tumour growth by sculpturing the immunogenic phenotype of tumours as they develop.[55] The question arises whether the immune system of decapod crustaceans is also capable of controlling and eliminating neoplastic cells, despite its fundamental structural and functional differences to the vertebrate immune system. Like in other invertebrates, the immune system of decapods includes only innate responses and no adaptive responses mediated by antibodies, which is typical of the vertebrates.[56–58] And even these innate immune systems differ significantly between vertebrates and invertebrates and also among the major invertebrate groups. For instance, the main characteristic of the arthropod immune system is melanization and encapsulation of pathogens and parasites by the pro-phenoloxidase activating system (proPO system) and the haemocytes.[56,57] The proPO system is similar in insects and crustaceans but the haemocytes differ among the 2 taxa. Moreover, in crustaceans the oxygen carrier haemocyanin, which is lacking in insects, can also exert prophenoloxidase activity.[56] In the Mollusca, pathogens are isolated and eliminated by haemocytic encapsulation as well but these capsules are not melanized.[58]

In the Decapoda, the immune system was shown to either phagocytose or melanize and encapsulate all kinds of foreign material including human tumour cells but also damaged areas of the own tissues.[29,56,59] The melanized capsules or nodules are composed of 2 layers, an amorphous inner melanin layer that originates from the interaction of the proPO system, the haemolymph and degranulating granulocytes, and an outer cellular layer that is composed of aggregated semigranular haemocytes (Fig. 2*a*).[29,56] The enclosed pathogens or cells are killed and disintegrated as a result of the cytotoxic action of quinones, the melanin precursors, and the physical shielding effect of the rigid melanin layer. Therefore, under the light and electron microscope, the content of a capsule is only recognizable during the melanization and encapsulation process (Fig. 2*b*) but not in the final stage. Consequently, melanized and encapsulated neoplastic areas would hardly be identified in tissues sampled arbitrarily from decapod species.

Although the probability is very low to detect signs of an immune defense against neoplastic cells in squash preparations or histological sections of decapods from the wild, there is evidence from one case that the decapod immune system can indeed eliminate neoplastic cells by phagocytosis and melanization and encapsulation. Sparks and Morado[24] presented a micrograph, which clearly shows a phagocytosed neoplastic cell within a granulocyte in *Paralithodes camtschaticus*. The authors further reported on histological evidence for attempts of granulocytes to encapsulate groups of tumour cells.[24] In insects, tumour cells are also melanized and encapsulated by the immune system as demonstrated by

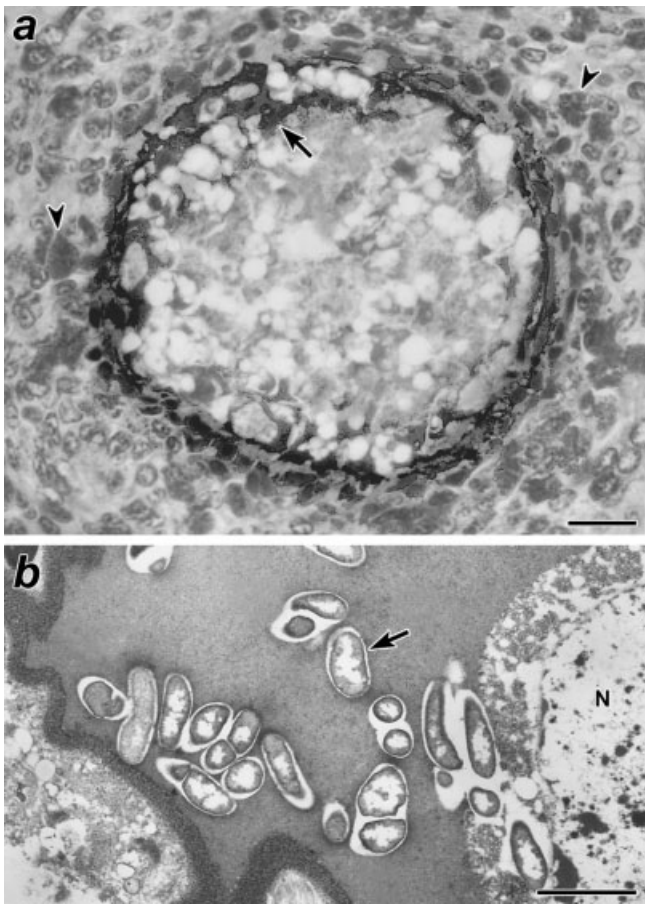

Figure 2 – Cytotoxicity of immune response in decapods. *(a)* Encapsulation and melanization of a bacteria infected tissue area in the hepatopancreas of the noble crayfish, *Astacus astacus*. The disintegrating tissue is enclosed by an inner melanin layer (arrow) and an outer layer of haemocytes, which is more tightly packed in the final stage of capsule formation. Arrowheads denote granular haemocytes, which include components of the proPO system. Light microscopy, Goldner staining. Bar: 20 μm. *(b)* Early stage of the melanization process, showing disintegration of cell organelles (N, nucleus) and bacteria (arrow) within the polymerizing melanin mass. Electron micrograph. Bar: 2 μm.

Nappi,[60] using the benign tumour of the adipose tissue of *Drosophila melanogaster* as a model. These examples suggest that the immune system of the Decapoda, and the arthropods in general, is principally capable of isolating and eliminating neoplastic cells or neoplastic tissue areas.

Age-related cancer, stem cells and telomerase activity

In humans and other mammals aging is accompanied by a rapid increase in cancer.[38,61–63] For instance, in the U.S.A. cancer rates for persons aged 50–64 years are 7 to 16-fold higher than rates for younger persons, and rates for persons aged 65–74 years are 2 to 3-fold higher than rates for persons aged 50–64 years.[61] In laboratory rodents, 30% and more have tumours at the end of their 2-3 year life span.[38,64] Age-related cancer is not restricted to mammals but is also found in lower vertebrates. For instance, in zebrafish, which has a mean life span of 3.5 years, the incidence of seminomas was shown to increase up to 50% by 2 years of age in males.[65] Ageing in vertebrates is associated with a number of events at the molecular, cellular and physiological levels that are assumed to promote carcinogenesis and subsequent cancer growth. Anisimov[62] summarized these events and presented hypotheses on the relationship of ageing and cancer.

Age-related cancer is at least partly caused by the same mechanisms that ensure longevity by maintaining genomic stability and replicative integrity *via* telomere length preservation.[62,66–68] The telomeres form the ends of the chromosomes and are shortened with each round of cell division. Loss of the replicative capacity of cells caused by telomere shortening can be counteracted by activation of telomerase, a reverse transcriptase, which adds new repeats to the ends of the chromosomes. In the adults of humans and other animals with determinate growth, the rapid growth to a mature conclusive size, the somatic tissues usually lack telomerase activity, whereas it is expressed in embryonic tissues and tumour cells.[67] In contrast, animals with indeterminate growth, *i.e*. growth that continues throughout the entire life span of an individual, express telomerase also in the tissues and organs of the adults as was shown for the rainbow trout, *Oncorhynchus mykiss*,[69] and the American lobster.[70]

In the Decapoda, there is only one report on a tumour in an old specimen, namely in the stomach wall of an American lobster,[4] although many decapod species can grow rather old. Some species like the American lobster and the Japanese spider crab, *Macrocheira kaempferi*, can even reach maximal life spans close to 100 years. I have searched for neoplasias in old specimens of the marbled crayfish,[71] which has a life span similar to mouse and zebrafish, but my efforts were without success. The virtual absence of age-related cancer in the Decapoda may be related to their negligible functional senescence,[72] which is in contrast to mammals[67,73] and insects.[74] It may also be linked to specific mechanisms that maintain stem cell integrity until the end of life, for example by telomere protection. Telomeres and telomerase activity in decapods were analyzed by using the American lobster as an example.[70] High telomerase activities were detected in all ages and all organs investigated. The highest relative telomerase value was measured in the hepatopancreas, the organ with the most rapid cell turnover in the Decapoda.[3]

Growth in decapod crustaceans is not a continuous process like in mammals because decapods enlarge their body step-wise by moulting. During ecdysis the rigid old exoskeleton is shed, and thereafter the body is enlarged by uptake of water. The new exoskeleton is then adjusted to this new size and is not significantly changed until the next ecdysis. In the intermoult period the water content of the tissues is successively reduced and replaced by the enlargement of new cells, which originate from the activity of stem cells or precursor cells. Such stem cells are obviously located in all tissues and organs and serve for growth and repair (Figs. 3*b*, and 3*c*). Most of these stem cells, for instance the myogenic satellite cells of the heart, are quiescent during intermoult but are activated after ecdysis for a short period of time.[71,75] The marbled crayfish passes through 20–25 moulting cycles in its lifetime, and accordingly, the moult-dependent stem cells are activated and silenced 20–25 times. In old lobsters, there are probably more than 100 moult-related divisions. However, it is not yet known whether an individual stem cell divides only once or several times during a growth pulse initiated by moulting.

The moulting cycle, and hence the activity of the moult dependent stem cell systems, is regulated by the moulting hormone ecdysone, as was shown for the myogenic stem cells in the heart of crayfish.[3,75] The steroid hormone ecdysone inclusive of its signalling pathway is rather well investigated. It is negatively regulated by the moult inhibiting hormone, which in crayfish has a molecular mass of 8,322 Da and a length of 72 amino acid residues.[3,76] Ecdysone production is stimulated by the terpenoid methyl farnesoate, a relative of the insect juvenile hormone.[77] Methyl farnesoate is negatively regulated by the mandibular organ inhibiting hormone, which is a peptide hormone of 77 amino acid residues.[3]

Some stem cell systems are independent from the moulting cycle, for instance the E-cells of the hepatopancreas, which divide in the final phase of each feeding cycle to replace cells that are

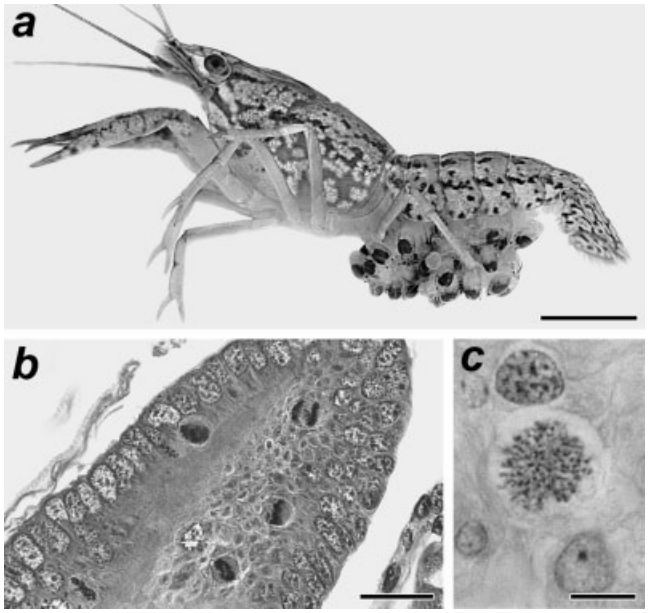

FIGURE 3 – The marbled crayfish and selected stem cell systems. (*a*) Breeding female carrying juveniles under the pleon. Bar: 1 cm. (*b*) Mitotic pulse in group of stem cells (E-cells) at blindly ending tip of a hepatopancreas tubule. Light microscopy, Goldner staining. Bar: 20 μm. (*c*) Mitosis of myogenic stem cell located individually in the ovarian envelope. Note high number of small chromosomes. Light microscopy, Goldner staining. Bar: 10 μm.

regularly discharged during processing of the food.[3] The hepatopancreas is the central organ of metabolism in the Decapoda and includes functions like absorption and metabolism of nutrients, storage of reserves, synthesis of digestive enzymes, and detoxification of xenobiotics.[3] The E-cell system is particularly interesting because these stem cells are not arbitrarily distributed as in other organs (Fig. 3*c*) but rather concentrated at the blindly ending tips of the numerous hepatopancreas tubules, propagating their descendants in one direction only (Fig. 3*b*). This particular division pattern results in the establishment of a distinct age gradient along the hepatopancreas tubules, which resembles the situation in the crypts of Lieberkühn of the small intestine of mammals[78] but even more the terminal meristems of plants. In the marbled crayfish, the E-cells are estimated to divide approximately 1,000 times in their 3-years life time, which closely resembles the stem cells of the small intestine of mouse.[78]

These and further rather well characterized stem cell systems of the decapod crustaceans such as the neurogenic stem cells of the brain,[79] the haematopoietic stem cells[80] or the stem cells and progenitor cells involved in regeneration of lost appendages[81] offer promising material to investigate the molecular mechanisms that govern activation and silencing of stem cells and maintenance of their functional integrity even in higher ages. Comparisons between decapods and vertebrates like mouse and zebrafish, which have similar body sizes and life spans but strikingly different tumour frequencies, may contribute to the understanding of age-associated cancer. Of particular interest is the comparison of decapods and the zebrafish, because both share characters such as indeterminate growth, high telomerase activity in the tissues and organs of the adults and low to negligible functional senescence[72,82] but differ strikingly with respect to age-related cancer.

Niches for decapod crustaceans in cancer research and availability of suitable laboratory models

Mammalian models, particularly the mouse, *Mus musculus*, and the rat, *Rattus norvegicus*, are the most important experimental animals in cancer research because of their physiological, cellular and molecular similarity to humans. They were used to identify carcinogenic substances, to analyse the molecular and cellular mechanisms of tumour formation and tumour growth, to identify the genes involved in tumour formation and suppression and to develop drugs for cancer treatment.[37,83,84] However, in the last 2 decades, non-mammalian animal models have become increasingly important to investigate particular aspects of carcinogenesis that are currently not well understood, resulting in the development of new concepts and technologies for cancer treatment.[34,36,85–89]

For instance, the zebrafish, *Danio rerio*, which can develop almost any tumour type known from humans, is suitable to investigate the signalling pathways during tumourigenesis, to image tumour progression in living fish by fluorescence and to manipulate tumour development with chemical compounds and genetically *via* enhancers and supressors.[36,85] The fruit fly, *Drosophila melanogaster*, contributed to investigation of cancer candidate genes, suppressors genes, and the development of cancer treatment by DNA methyltransferase inhibitors.[34,86–88] And the nematode, *Caenorhabditis elegans*, served as a model to investigate the role that the phylogenetically highly conserved cancer related genes play in normal signalling pathways of developmental processes.[89]

The decapod crustaceans appear suitable to occupy another niche in cancer research, namely the investigation of mechanisms that prevent or control spontaneously and environmentally induced cell proliferation. Of particular interest are the pathways that keep the degree of DNA adduct formation with chemical carcinogens low, that prevent uncontrolled proliferation of stem cells even in higher ages, and that eliminate neoplastic cells from the tissues and circulation. Decapod crustaceans may also be suitable to investigate the role of epigenetic mechanisms in tumour control. It is well known that an altered pattern of epigenetic modification is central to human cancer, *e.g.* the transcriptional silencing of tumour suppressor genes by CpG-island-promotor hypermethylation.[90] In contrast to *C. elegans* that lacks methylated DNA and *Drosophila* that possesses methylated DNA only through the embryonic stages, decapod crustaceans have methylated DNA also in the adult life-stages.[91] The marbled crayfish, for instance, has global DNA methylation values of ~1.5%–2.1%,[91] which corresponds to roughly half of the values in humans.[90]

Cancer-related genetics, and genetics in general, is not as developed in the decapod crustaceans as it is in mouse, zebrafish, *Drosophila* and *Caenorhabditis*. At present, there is no complete DNA sequence of a decapod species available but the shrimp genomics research community is presently producing the necessary elements required to develop a full genome sequencing project, *e.g.* EST databases, linkage maps or BAC libraries.[92–94] For instance, for the most valuable commercial shrimp, *Litopenaeus vannamei*, there are already 155,411 ESTs in the NCBI database (http://www.ncbi.nlm.nih.gov/sites/entrez?db=nuccore) and for the kuruma shrimp, *Marsupenaeus japonicus*, the number of collected clones for a BAC library is ~190.000, covering >3 times the haploid genome of 2.000 Mb.[93] The Decapoda have a genome size ranging from 1.07 pg in the common shore crab, *Carcinus maenas*, to 40.89 pg in the Arctic shrimp, *Sclerocrangon ferox* (http://www.genomesize. com/).[95,96] Their chromosome numbers are relatively high when compared with other animal taxa (Fig. 3*c*). The penaeid shrimp and crayfish, for instance, have haploid chromosome numbers of approximately 44 and 100, respectively. The Decapoda even include the animal record holder in chromosome number, namely *Pacifastacus leniusculus trowbridgii* with $n = 188$.[95] Investigations on the identification of particular genes or their products, for instance by cDNA microarrays, are under way.[97,98]

Many proto-oncogenes and tumour suppressor genes are highly conserved among the animals and even the Eukaryota[87,99] and may also be found in the Decapoda. Moreover, since the Crustacea and Insecta are taxonomical sister groups[100] many homologous genes are expected to be shared by them, and thus, cancer-related

genetics in the decapods may greatly profit from the extensive genetic knowledge in *Drosophila*. Some tumour suppressor homologues of humans were already identified in decapod species. For instance, Xu *et al*.[101] found a homologue of the putative tumour suppressor QM in *Marsupenaeus japonicus*, which was shown to be involved in regulation of phenoloxidase activity, a main step of the immune response of decapod crustaceans. In humans decreased QM expression was associated with early development of prostate cancer.[102]

A further tumour suppressor-like protein (TSL), which has ~60% identity with the human tumour suppressor OVCA1 (ovarian cancer 1)[103] was isolated from a penaeid shrimp infected with the white spot syndrome virus (WSSV). Transient expression of TSL in cultured baby hamster kindney cells led to apoptosis, which was rescued by the viral anti-apoptosis protein WSSV222.[104] Moreover, shrimp TSL has been shown to play a tumour suppressor role when expressed in the human ovarian cancer cell line A2780. Another homologue related to human cancer is shrimp fortilin, which was found in the black tiger shrimp, *Penaeus monodon*.[105] This protein has 64% identity in amino acid composition with human fortilin, also known as translationally controlled tumour protein (TCTP). Human fortilin is an anti-apoptotic molecule that is overexpressed in many cancers.[106] Shrimp fortilin can protect cells under toxic conditions from death and can exert this function even when overexpressed in mammalian cells, suggesting that shrimp and human fortilin use a common cellular pathway.[105]

Decapod species that could serve as experimental animals for cancer research are at hand because many species of the shrimp, lobsters, crayfish and crabs have a long tradition of culture in the laboratory.[107] These experimental animals could easily be exposed to nutritional and water-borne carcinogens and could be sampled for investigation of the detoxification pathways and other cancer-related topics like stem cell regulation and anti-tumour function of the immune system. Production of transgenic decapods for cancer research is also principally possible as shown by Sarmasik *et al*.[108] and Yazawa *et al*.[109] who have successfully transferred genes into the shrimp *Penaeus monodon* and the freshwater crayfish *Procambarus clarkii* by different methods such as retroviral vector technique, microinjection and particle gun bombardment. Stem cells of decapods can not only be investigated *in situ* but also *in vitro*. For instance, a crayfish haematopoietic cell line is well established and routinely used to replicate crustacean viruses and to study host-virus interactions.[110] Further literature on cell and tissue culture in decapods is found in Uma *et al*.[111]

A particularly promising decapod species for cancer research is the parthenogenetic marbled crayfish (Fig. 3*a*),[112] which is presently being introduced as a laboratory model for development and epigenetics.[71] This crayfish is characterized by direct development, a generation time of approximately 6 months and a life span of ~3 years,[113,114] which is similar to that of mouse and zebrafish. It can produce up to 400 progeny per batch, which are genetically identical to their mother and among each other as shown by the microsatellite technique.[85,115] The eggs and first juvenile stages are carried under the pleon (Fig. 3*a*) but can also be individually raised *in vitro*.[32,114] All life stages are easily accessible, are tolerant to physical manipulation and are amenable to all kinds of experimental manipulation. These features and the ability of all life stages to thrive on a single pellet food in very simple housing systems, including micro-plates for the embryonic and first postembryonic life stages,[32,114] facilitate performance of highly standardized experiments and precise sampling. All of the analytical techniques developed for shrimp, lobsters, crabs and crayfish are principally adoptable for the marbled crayfish.

Conclusion

It was the aim of this paper to review the state of the art of neoplasias in decapod crustaceans and to direct the attention of the cancer research community to the anti-cancer research potential of this animal group. The Decapoda are significantly less affected by tumours than mammals, fish, insects and bivalves although they have comparable life span and are exposed to similar carcinogenic impacts from the environment and the food. The low rate of carcinogenesis in decapods is apparently related to peculiarities of their detoxification pathways and their immune system and to specific mechanisms that ensure integrity of their stem cells throughout life. Examples are the rapid elimination of PAH-related DNA adducts from the tissues, phagocytosis or encapsulation and melanization of neoplastic cells by the immune system, and maintenance of high telomerase activity in the tissues throughout life. In-depth investigation of these topics and comparison with the cancer models mouse, zebrafish and *Drosophila* is expected to provide new information on how an organism can successfully prevent tumour formation. Suitable decapods that could be used as laboratory models are at hand and tools for research on genomics and gene expression are presently adopted for the decapod crustaceans. A particularly promising model to study cancer resistance in the Decapoda is the marbled crayfish, a new model for development and epigenetics that produces high amounts of genetically identical offspring. Knowledge obtained by research on the mechanisms of cancer prevention in the decapod crustaceans might open new windows in the fight against cancer in humans.

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