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TUMORIGENESIS AND TUMOR MARKERS IN FISH: AN UPDATED REVIEW

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ABSTRACT

Fish is the largest class of vertebrates. Neoplasia is a known disease in fishes which is not considered to be a deadly condition for few malignancies. Cancer is the unregulated cell proliferation with the ability to invade or metastasize to other parts of the body. The tumorigenic process is marked by a competition between cellular proliferation and apoptosis characterized by genetic mutations and increased connective tissue proliferation with associated impairment of the immune system. Tumor markers are molecules produced by tumor cells or other cells of the body in response to cancer or benign conditions and thus; are of diagnostic value. Most tumor markers are secreted into blood and are assayed in blood, urine or immuno-histochemically. Diagnosis of tumors in fish is not always easy to carry out, and the tool provided by antibodies used on mammalian tissue is essential for obtaining definitive, unambiguous, and inexpensive identification. Generally, teleosts resemble other vertebrates in their predisposition to neoplastic lesions, which been widely studied, characterized, and classified. Immunohistochemistry is an extremely useful technique for the diagnosis of neoplasms. This technology relies upon antibody-based stains specific for intermediate filaments or surface markers. This review discusses the currently available tumor markers for different neoplasms in fish as well as the mostly used tumor markers in diagnosis. The objective of this review also is to provide an overview of neoplasia and the various neoplastic disease conditions in fishes as well as the most common methods used in diagnosis of fish tumors including immunohistochemistry and tumor markers.

Keywords: Fish; tumor; neoplasia; markers; tumorigenesis; diagnosis.

INTRODUCTION

Fish is filled with omega-3 fatty acids and vitamins such as D and B2 (riboflavin). Fish is rich in calcium and phosphorus and a great source of minerals, such as iron, zinc, iodine, magnesium, and potassium. The American Heart Association recommends eating fish at least two times per week as part of a healthy diet. Fish is a low-fat high quality protein. Fish is filled with omega-3 fatty acids and vitamins such as D and B2 (riboflavin). Fish is rich in calcium and phosphorus and a great source of minerals, such as iron, zinc, iodine, magnesium, and potassium. The American Heart Association recommends eating fish at least two times per week as part of a healthy diet. Fish is packed with protein, vitamins, and nutrients that can lower blood pressure and help reduce the risk of a heart attack or stroke.

Eating fish is an important source of omega-3 fatty acids. These essential nutrients keep our heart and brain healthy. Two omega-3 fatty acids found in fish are EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid). Our bodies don't produce omega-3 fatty acids so we must get them through the food we eat. Omega-3 fatty acids are found in every kind of fish, but are especially high in fatty fish. Some good choices are salmon, trout, sardines, herring, canned mackerel, canned light tuna, and oysters (Fig. 1).

Omega-3 Fatty Acids: Help maintain a healthy heart by lowering blood pressure and reducing the risk of sudden death, heart attack, abnormal heart rhythms, and strokes. Omega-3 fatty acids Aid healthy brain function and infant development of vision and nerves during pregnancy. It may decrease the risk of depression, ADHD, Alzheimer's disease, dementia, and diabetes. May prevent inflammation and reduce the risk of arthritis.

History of Cancer and Carcinogenesis

"Cancer is a fatal disease caused by uncontrolled proliferation of the cell (s) due to, genetic mutations which may be inherited or acquired due to DNA damage and or epigenetic alterations (Fig. 2a). Cancer is as old as the pre-human itself. Evidence of cancer cells was found in dinosaurs which date back to 70-80 million years ago. The human cancer was first reported in the scripting of Edwin Smith papyrus roll and Ebers Papyrus. Generally, tumors can possess ten to thousands of mutations, but few of them are as the main cause of cancer progression" [1]. The disrupted cells escape normal regulatory mechanisms and appear abnormal in function and structure [2]. The tumorigenic process is marked by a competition between hyperplasia and apoptosis characterized by genetic mutations and increased connective tissue proliferation (Fig .2b). Three distinct stages of carcinogenesis (tumorigenesis) are the initial proliferation, hyperplasia and full sized tumor stage [3]. Tumor (neoplastic mass) consists of abnormal and un-regulated proliferating cells of parenchymatous or connective tissue origin with vascular supply required for cancer nutrition, growth, progression and metastasis. Cancers may be of epithelial cells origin (carcinomas) as seen in the lungs, skin, breast, liver and pancreas or of mesenchymal origin (sarcoma). Genetic alterations promote progression such as hyperplasia and proliferation that leads to abnormal morphology, as well as cell disorganization [3]. Malignant tumor has several phenotypic features such as excessive growth, local invasiveness, un-differentiation of cells and the ability to form distant metastasis. These characteristics are in a stepwise fashion phenomenon called tumor progression (Fig. 2c). "The importance of neoplasm lies in their effects on the host. Any tumor, even benign ones, may cause morbidity and mortality. Cancers are more threatening to the host than benign tumors. Nevertheless, both types may cause problems such as effects on functional activity like hormone synthesis, hemorrhage and secondary infection induced in case of ulcerated neoplastic lesions. Cancer also may cause wasting (cachexia) or para-neoplastic syndromes" [4]. Cancers results from multisteps processes, causing a transformation of the normal cell into malignant ones (Fig. 2 a, b, c/Table 1) and also give a chance for the pre-cancerous lesions to become malignant lesion.

"Keap1-Nrf2 one of the most important signaling pathway relating to antioxidant and tumorigenesis, and its abnormal activation is related to cancer metastasis and drug resistance. $Nrf2^{Thr80}$ and $Nrf2^{Pro85}$ play a vital role in the Keap1-Nrf2 interaction. Mutant or

Table 1. Different types of carcinogens [5]

Carcinogens	Example
1-Physical carcinogens	UVR, Ionizing radiation
2-Chemical carcinogens	Asbestos, Tobacco, Alcohol, Arsenic, Aflatoxin (food)
3-Biological carcinogens	Virus ,bacteria or parasites
4-Agening from another fundamental factors	

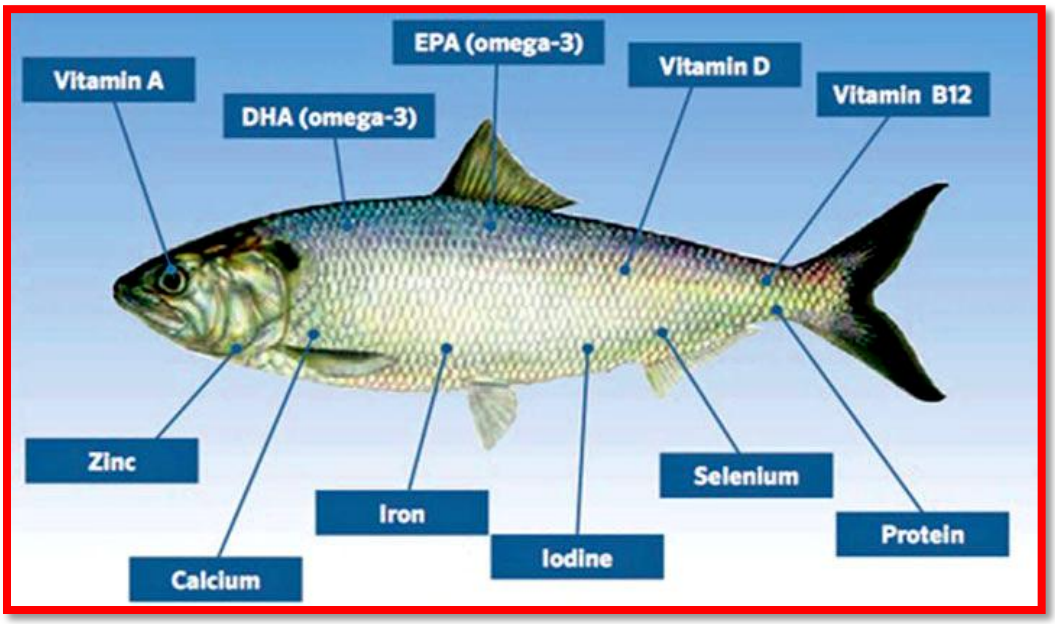

Fig. 1. Nutritional values of fishes

modification at position Thr80 will disrupt the interaction. Especially, $Nrf2^{Thr80}$ and $Nrf2^{Pro85}$ mutations activate the expression of cytoprotective genes in HEK293T cells. As for Keap1, except G364C, the binding affinity of other cancer-related mutants to Nrf2 hardly changed, which means that Keap1 mutants can activate Nrf2 without disrupting the binding to Nrf2" [6].

Fish Neoplasia

Fish develop neoplasia or cancer just like higher animals. All teleost fishes in any part of the world could potentially develop neoplasia. In bony fishes, neoplasms of the connective tissues, such as fibroma and fibrosarcoma, are most common. "For unknown reasons cancer has been rare in cartilaginous fishes such as sharks and rays. Known and suspected factors contributing to neoplasia in fish include viruses, environmental chemicals (carcinogens), repeated physical trauma, hormones, age, sex, genetic predisposition and immunological competence of the host. Generally, neoplastic growths are spontaneous within an individual due to

congenital malformation, age or genetic predisposition but could also be caused by environmental conditions. Neoplasms usually become apparent by gross observation of an external or internal swelling, lump, or formation of an abnormal tissue growth. Except for neoplasia caused by infectious viruses, horizontal fish to fish transmission does not occur" [7]. Neoplasms in fish as well as the others are classified according to the cell or tissue of origin and are further grouped based on benign or malignant characteristics (Table 4). "Benign tumors are often well-differentiated, grow slowly, are well circumscribed without invading surrounding normal tissue and do not metastasize. Most benign neoplasms are not usually life threatening and often end in the suffix "oma". Exceptions are benign neoplasms of the brain and some endocrine organs that can be life threatening due to their location and deleterious physiological effects on the host. Malignant tumors are often not well differentiated, may grow rapidly, infiltrate normal tissues and tend to metastasize [7]. The names of these neoplasms are often preceded by the word "malignant" or with the suffixes "sarcoma" or "carcinoma" [7].

Table 2. Types and classification of cancer in fish [7]

Tissue type	Benign tumors	Malignant tumors
Epithelial	Papilloma	Epithelial carcinoma
	Adenoma	Adenocarcinoma
Mesenchymal	Fibroma(CT)	Fibrosarcoma
	Leiomyoma(SM)	Leiomyosarcoma
	Rhabdomyoma(St.M)	Rhabdomyosarcoma
	Lipoma(Fat)	Liposarcoma
	Chondroma(Cart)	Chondrosarcoma
	Oesteoma(Bone)	Osteosarcoma
Hematopoietic	Lymphoma	Lymphosarcoma
Blood vessels	Hemangioma	Hemangiosarcoma
Nerve cells/N crest	Schwannoma	Glioma-Astrocytoma
Pigment	Erythrophoroma	Malignant melanoma
Embryonal	Nephroblastoma	

Table 3. The most common fish tumors in relation to organs and species

Tumor	Fish species	Organs	Reference
Hemangioendtheliosarcoma	Rainbow trout (RT)	Operculum, muscles	Barbara and Ruth, [8]
Melanoma	RT , Catfish	Surface skin barbles fins	Barbara and Ruth, [8]
Malignant Melanoma	Chinook salmon	Muscles	[9]
Thyoma	Adult trout	Operculum	[9]
Adenocarcinoma	Golden RT	Kidney	Barbara and Ruth, [8]
Nephroblastoma	Adult RT	Kidney	[9]
Fibrolipoma	Adult wild RT	Skin & Adipose tissues	Barbara and Ruth, [8]

Tumor	Fish species	Organs	Reference
Malignant lymphoma	Salmon	Gills and operculum	Barbara and Ruth, [8]
HCC	All species	Kidney &liver	[9]
Metastatic HCC	All species	Kidney	[9]
Skin neoplasms	Asian Sea bass	Skin & muscles	[9]
Ovarian neoplasms	Sea bass	Ovary	[9]
Adenoma	Catfish	Swim bladder	[9]
Fibroma	Catfish& Angel fish	Skin &lips	[9]
Fibrosarcoma	Tilapia	Skin	[9]
Hemangioma	RT	Subcutaneous Blood vessels	[9]
Thyroid Adenoma	RT -Tilapia	Skin & gills	[9]
Pancreatic Adenocarcinoma	RT -catfish-tilapia	Liver & pancreas	[9]
Lymphosarcoma	Tilapia	Ovary	[9]
Monocytic leukemia	All species	Liver	[9]
Pigment cell tumors (Erythromas ,Irridophoromas ,and Melanophoromas)	Golden fish	Skin & neural crests	[9]
Ameloblastoma	King salmon	Teeth	[9]
Brickle cell carcinoma	Sablefish	Epidermis	[10,11]
Fibrosarcoma	Sock eye salmon Salmon	Muscles, body wall	[10,11]
Rhabdomyosarcoma	Pacific halibut	Striated muscles	[10,11]
Black melanoma	Chun salmon	Head	[12]
Pedunclated papilloma	Coho salmon	Back	[12]
Liposarcoma	Rock fish White fish	Back & abdomen	Lionel and Thomas, [13]
Fibroma	Sock eye salmon	Dorsal flank	[9]
Thymic lymphosarcoma	White fish	Gills	[9]

“Fishes develop tumors and cancers, much like humans and other animals. Most fishes get tumors or cancers due to genetic predisposition. Some fishes, however, can get tumors or cancers from a viral infection. Most tumors are seen as bumps or lumps under the fish's skin. But the location and signs of the tumor can be different for each fish, and depend greatly on the type of tumor. The fish’s ability to eat and swim will be affected, causing a rapid decline in its health. Koi fishes usually get tumors in the reproductive organs. They will have swollen abdomens and the illness can become terminal. Conversely, goldfish are susceptible to fibroma tumors and sarcoma cancers. While Gypsy-swordtail fishes, generally develop skin cancer (malignant melanoma).Another type of tumor is found in the gills. It causes the fish to be unable to close its gills, and is due to a thyroid dysfunction. Despite its seriousness, the tumor has a good success rate when it is treated”.
(https://www.adfg.alaska.gov/static/species/disease/pdfs/fishdiseases/neoplasia.).

Prognosis for fish having neoplasms depends on the type of tumor and whether the lesion is benign or malignant. Benign

tumors are usually not life threatening. Malignant tumors can cause mortality if growth is rapid and interferes with normal organ functions. “Although aesthetically disturbing, there are no direct human health concerns associated with neoplasia in fish. Neoplasia is generally a rare event affecting one fish in several thousand. Should tumors occur more frequently in a population of fish, an indirect human health concern would be whether the cause is linked to environmental contamination” [14]. “Fish is the largest class of vertebrates. Neoplasia is not an uncommon disease in fishes; however, neoplasia in fishes is not considered a dangerous condition with relatively few exceptions of malignant disease” [11]. “Various types of neoplasms involved in several tissues (organs) have been recognized and described in different freshwater as well as marine fish species” [15-17]. A tumor will persist even if the inciting cause is eliminated [18]. Tumors are life threatening and sometimes not. They are classified into categories according to the behavior “benign” tumors that are usually (but not always) less dangerous and not aggressive to the host, and “malignant” tumors, those that are more likely cause to the host some degrees of harm if not death [12]. “The incidence of neoplasms frequently is higher in some fish species and in some different geographic areas. Neoplastic diseases similar to those found in other animals are found in fish. Some tumors are occurred due to genetically abnormalities mediated, such as the malignant melanoma of the Swordtail, and possibly the pseudobranch tumor of cod, thyroid tumors, malignant lymphosarcoma of northern pike, and fibromas or sarcomas of goldfish. Liposarcomas have been reported in captive-bred clownfish, and pigmented tumors of unknown origin have been seen in wild Hawaiian marine butterfly fishes, *Chaetodon miliaris* and *C multicinctus*. Both species are popular in the marine aquarium hobby. Although the reported incidence of tumors in sharks, skates, and rays is low, neoplasia does occur”. Barbara and Ruth [8] “Ovarian tumors are important neoplastic disorders of many types of fishes and have been reported in koi and northern pike. Typically, fish present with a swollen abdomen, and, depending on the severity of disease, there may be significant loss of condition. Viruses, especially retroviruses, have been associated with neoplasia in fishes. Two examples of viral-induced neoplasia have occurred in tropical fishes. Viral particles have been found in fibromas on the lips of freshwater angelfish. Debulking the tumor can allow affected fish to feed normally. Bicolor damselfish neurofibromatosis, a fatal disease, was reported in 1991 in wild *Stegastes partitus* found on the reefs of south Florida; it is believed to be a viral-induced tumor. Although not reported in captive bicolor damselfish, it has the potential to occur” Barbara and Ruth [8]. “Three basic pigmented cells showed in fish, melanocytes (melanin-producing cells), erythrophores (red or yellow pigment cells), and iridophores (iridescence-producing cells), are derived from neural crest. Tumors of pigment cells in fish are melanomas, erythrophoromas, and iridophoromas. These pigment cell tumors are among the most common types in bony fish and seem to be more common in fish than in mammals, including humans” [10].

“There are strong relationship between the immune system in fish and neoplasia, invasive malignancy and tumor immunity in invertebrates and no mammalian vertebrates. Again complex interactions between neoplasia and the host immune system was detected” [19]**.** Histologic features of tumors are extremely useful in

determining the differences between the benign or malignant tumors, if it still locally or metastatic spreaded. Whether or not it has been completely removed during a surgical biopsy, and what the overall prognosis is for the host [18]. Benign tumors tend to be less dangerous in spite of some tumors leads to death of fish. The gross and histologic features that characterize a benign tumor include: Table 4. The similarity to the cells/tissue of origin: Well differentiated: the tumor cells show ordered maturation and well defined differentiation; nuclear to cytoplasmic ratio is appropriate; few, if any, mitotic figures; cell products are formed at proper location; Encapsulated: these tumors frequently, but not always, are bordered by a discrete fibrous capsule; Expansile: these tumors enlarge, compressing adjacent normal tissue; Non-invasive: the tumor remains confined with no penetration into adjacent normal tissue; No metastasis: the tumor does not spread by way of the circulatory system or by implantation across body cavities. Malignant tumors tend to be more serious in terms of biological consequence to the host. Many malignant tumors will eventually cause the death of the patient by various means, although some can be less aggressive and may not have a serious impact on long term survival. Histologic characteristics of malignant tumors are: Differentiation varies: some malignant tumors are very well differentiated, looking very similar to the tissue of origin; others may show some similarity, while others have no resemblance to the tissue of origin; Capsule varies: while some may be weakly encapsulated, most lack this feature; Invasive: these tumors frequently invade into adjacent normal tissue; Metastasis: the tumor spreads by invasion of the vasculature and release of tumor emboli that spread to distant sites, or tumor cells spread across body cavities and implant on adjacent organs or surfaces; by definition, a metastatic tumor is malignant; metastasis is not commonly reported in fish; Necrosis: malignant tumors often grow so rapidly that they outgrow their blood supply; Cellular anaplasia: this is the failure to differentiate; the cells may be pleomorphic, may have larger than normal or misshapen nuclei [18].

The importance of neoplasms lies in their effects on the host. Any tumor, even a benign one, may cause morbidity and mortality. Cancer is far more threatening to the host than benign tumors. Neoplasms can't be enlarged behind 1-2 mm in diameter (as maximum distance), as both oxygen and nutrients can be infiltrated the blood that needed for tumor cell growth and proliferation. Cancer must be a mass of rapidly and permanently dividing cells. Malignant transformation results from mutation of oncogenes, tumor suppressor genes apoptosis regulating genes. There are many correlation between angiogenesis (neovascularization) and carcinogenesis (tumor production) such as:- angiogenesis supplies both blood oxygen and nutrients for the tumor mass, the newly formed endothelial cells initiate the growth of the cells around the neoplastic cells through producing some of an important polypeptides such as insulin-like growth factors, platelet-derived growth factor (PDGF), granulocytes-macrophage colony-stimulating factor (GM-CSF) and interleukin-1 (IL-1). Finally, angiogenesis is an important facilitator of metastasis [13]. “Ancillary techniques, such as Immunohistochemistry, as a valid diagnostic tool in the field of fish neoplasia. The immunohistochemical patterns are observed in four neoplasms in four different species. *Cytokeratin, vimentin, actin, S100, calretinin, and Melan-A antibodies* were used. Diagnoses of papilloma in a fresh water bream *(Abramis brama)*, fibroma (Fig. 3) in a

Sand Steenbras (*Lithognathus mormyrus)*, schwannoma in a Crucian Carp *(Carassius carassius)*, and melanoma in a spontaneously inbred *Xiphophorus* hybrid were made" [20]. "Although immunohistochemical identification (Fig. 4) is a technique that has been used for decades, it still remains an effective

Table 4. Differences between benign and malignant tumors [12]

Benign tumor	Malignant tumors
Well differentiated	Ill differentiated
Not aggressive	Aggressive
Encapsulated	Not encapsulated
Not spread	Local and distant spread
No recurrence after surgical removal	Recurrence after surgical removal
Well circumscribed	Ill circumscribed
No or few mitotic figures	Numerous mitotic figures
No features of anaplasia	Numerous features of anaplasia

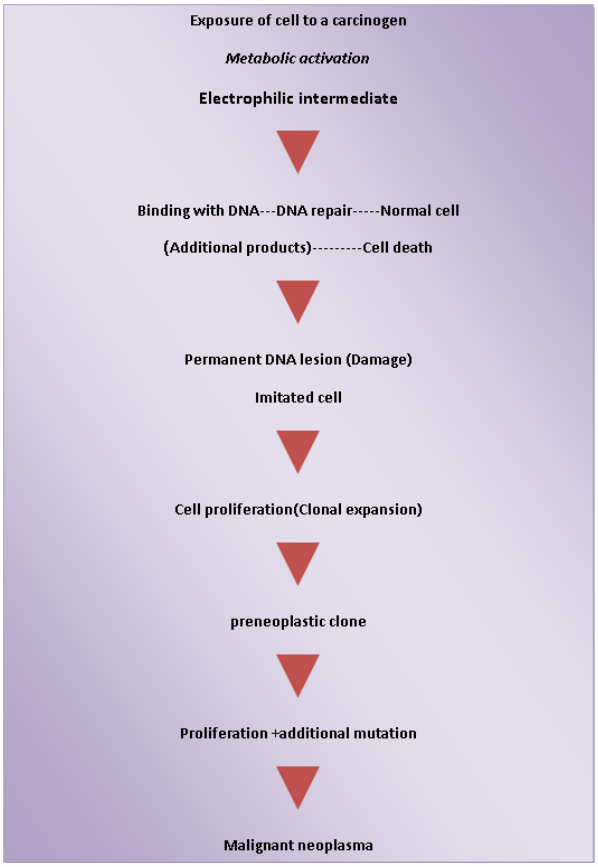

Fig. 2a. Molecular basis of cancer

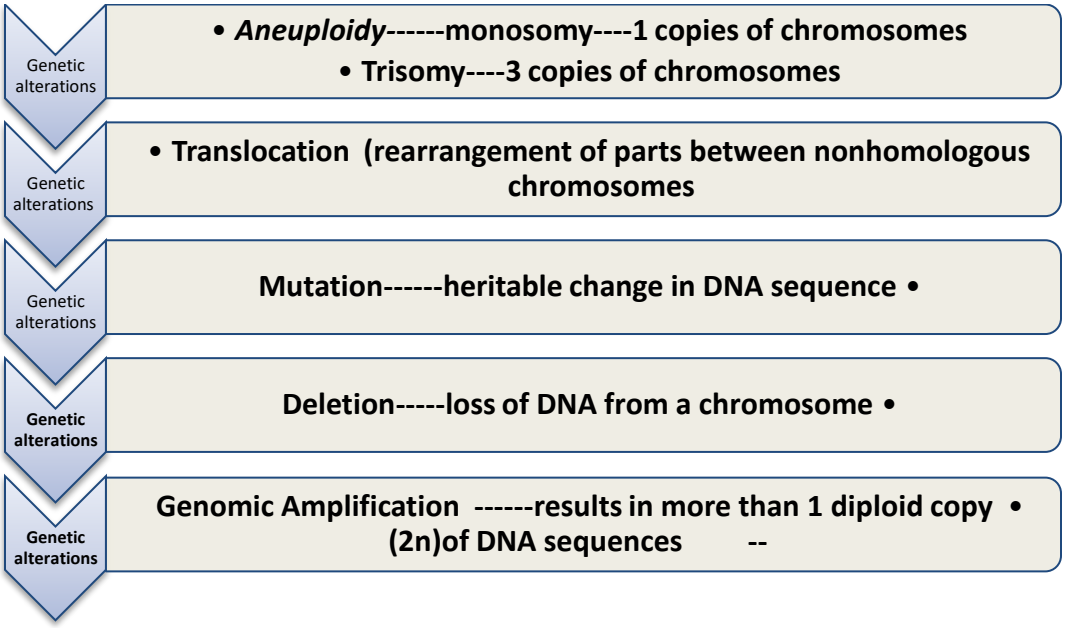

Fig. 2b. Tumorigenesis and Genetic mutations

diagnostic method in the field of neoplasia" [21,22].

Tumor Markers (TM) and Important Tumor Markers in Fishes

"Tumer Markers (TM) are molecules produced by tumor cells or other cells of the body in response to cancer or any certain benign conditions. Most tumor markers are secreted into blood and may be estimated in the blood, but they may also be measured in urine and tissues. An ideal tumor marker should have high sensitivity and specificity, should be cheap, and should be easily detected in body fluids. TM may be used for diagnosis, staging, and prognosis of cancer; they may also be used for monitoring treatment response as well as to check for cancer recurrence" [18]. There are a large number of tumor markers which are used for different types of cancers (Tables 5-7); many TM may also be elevated in more than one type of cancer. "Identification of novel markers is important and it is expected that with the advent of newer technologies, more reliable markers will be discovered" [18].

There are different ways of classifying cancer markers [23]. One traditionally accepted way of classification is into:-

1-Oncofetal antigens (CEA, AFP)
2-Glycoprotein antigens or carbohydrate antigens (CA 125, CA 19.9, CA 15-3)
3-Enzymes (PSA, ALP, NSE)
4-Hormone receptors (ER, PR)
5-Hormones (β-hCG, calcitonin)
6-Other biomolecules (VMA, 5HIAA)
7-Biochemical structure
8-Function
9-Combination of biochemical structure and function, and

10-Discovery of onco-fetal antigens

"An ideal tumor marker should have high sensitivity and specificity" [24]. "However, in practice the sensitivity and specificity of individual markers may vary widely. Tables 5, 6, 7 gives the sensitivity and specificity of some common markers. The drawbacks of available tumor markers [25] are: - Early detection is difficult, since low levels are seen in normal individuals. Large volume of cancer needed for significant elevation above normal. Some people with cancer never get elevated levels. Elevated levels may be seen in non-cancerous conditions".

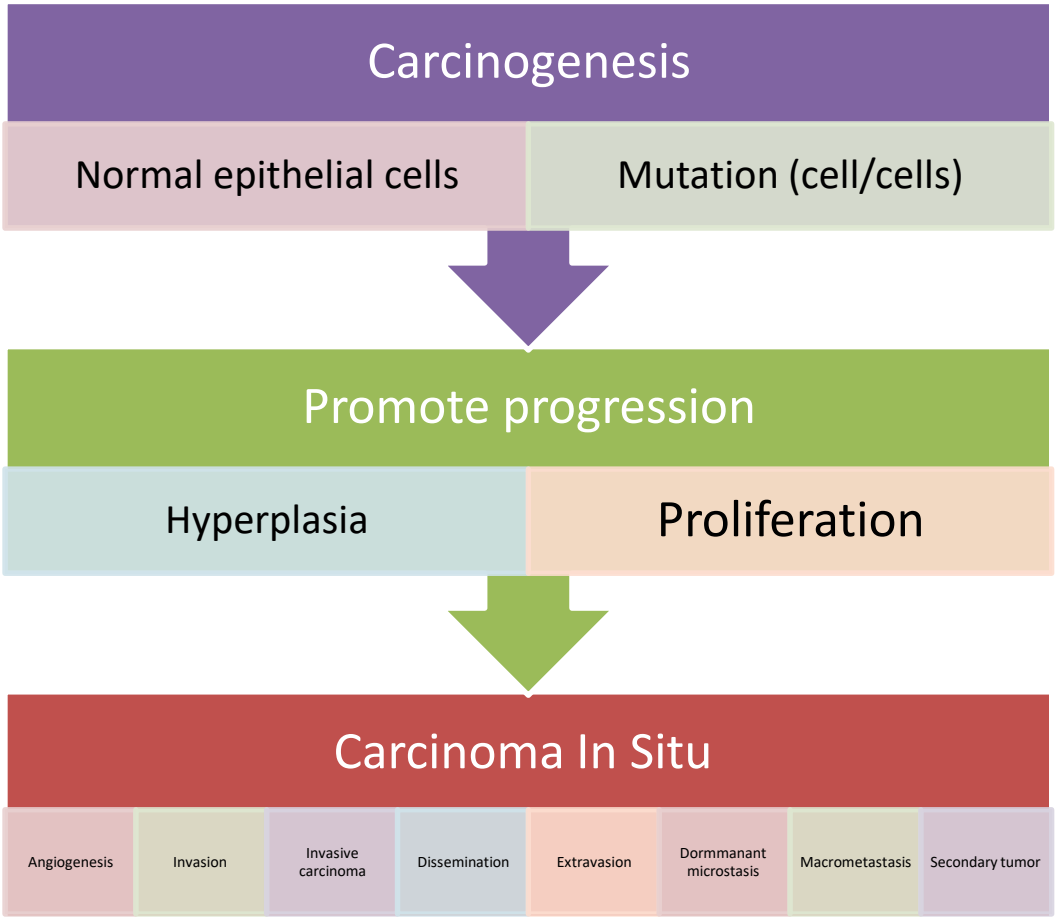

Fig. 2C. Cancer progression [3]

Table 5. Common used tumor markers for primary, secondary tumors as well as non neoplastic cases [18]

Tumor marker	Primary Ts	Secondary Tumors	Other cases
CA	Breast	Colon, lung, liver and stomach neoplasms	Ovarian .kidney diseases
CEA	Colorectal	Breast, lung, stomach, Pancreatic neoplasms	Peptic ulcer, cigarette smoking
CA	Pancreas	Colon, esophagus, liver	Pancreatitis, liver cirrhosis
AFP	HCG, GCT	Stomach, pancreas, biliary	Pregnancy, viral hepatitis
&-hCG	Non-seminomatous germ cell tumors. GIT, trophoblastic tumors	Rarely GIT cancers	Hypogonadal status marijuana
CA 125	Ovarian	Endometrium, fallopian tube Breast, lung, stomach	Menstruation, pregnancy, fibroids, ovarian cysts, pelvic inflammation
PSA	Prostate	None	Prostatitis, prostatic hypertrophy
CA50	Kidney	Nil	Mucin-like cancers
TPA/CA19.9	Bladder	Nil	Polypeptide antigen
CEA	Lung cancer Adenocarcinoma	Nil	----------
CYfra21.1	Epidermoid cancer/lung	Nil	Neuron specific enolase
AFP	Ovarian cancer Choriocarcinoma	Nil	----------
&-hCG	Ovarian cancer Choriocarcinoma Hydatidiform mole	Nil	----------
CA125	Serous cancer-ovary	Nil	----------
CEA	Mucinous cancers-ovary Adenocarcinoma (uterine) Colorectal cancers	Ni Nil CA 19,9 ,TPA	-------- ---------- ----------
CA19.9,CA72.4	Gastric cancer	CEA	----------
CEA.TPA,SCC	Esophageal cancer	Nil	----------
AFP, &-hCG	Testicular cancers	Nil	----------
PSA &PAP	Prostate cancers	Nil	----------
CA 19.9	Pancreatic cancer	CEA	----------

Table 6. According to types and pathogenesis TM in breast cancer

Use	Tumor markers
Commonly employed	CA 15-3 CA 27-29 CEA
Estrogen and Progesterone receptors(ER ,PR)	HER-2/NEU uPA PAI-1
Multi-pancreatic gene expression assay ,other potential markers are	DNA ploidy (Flow cytometry) P53 Cathepsin D Cyclin E
Proteomic markers	CTC
Bone marrow micrometastasis	Ki-67 P27 P21 Thymidine kinase Tropoisomerase II
Breast Cancer	BRCA1 &BRCA2
PR &ER	Breast cancers

Table 7. Markers and different types of tumors in different fish species

Marker	Tumor	Fish	Reference(s)
Cytokeratins	Epithelial tumors (e.g., papilloma)	Bream	Lanteri et al. [26]
Vimentin	Mesenchymal Tumors(e.g., fibroma)	Sand steenbars	[27] [28,29]
Calretinin (29-KDa)	Used to distinguish between Schwannoma and neurofibromas	Crucian Carp	Marino et al. [30]
S100 proteins	Schwannomas Ependymomas Astrogliomas (benign &Malignant Langerhans cells Interdigitating Reticular cells	Fish	Germanà et al. [31]
Melan-A	Malignant melanomas, Melanocytic nevi	Fish	Pitcovski et al. [32]
CD3 and CD79	Used to differentiate T lymphocytes from B-lymphocytes in cases of lymphosarcoma	Fish	Sonia et al. [12]

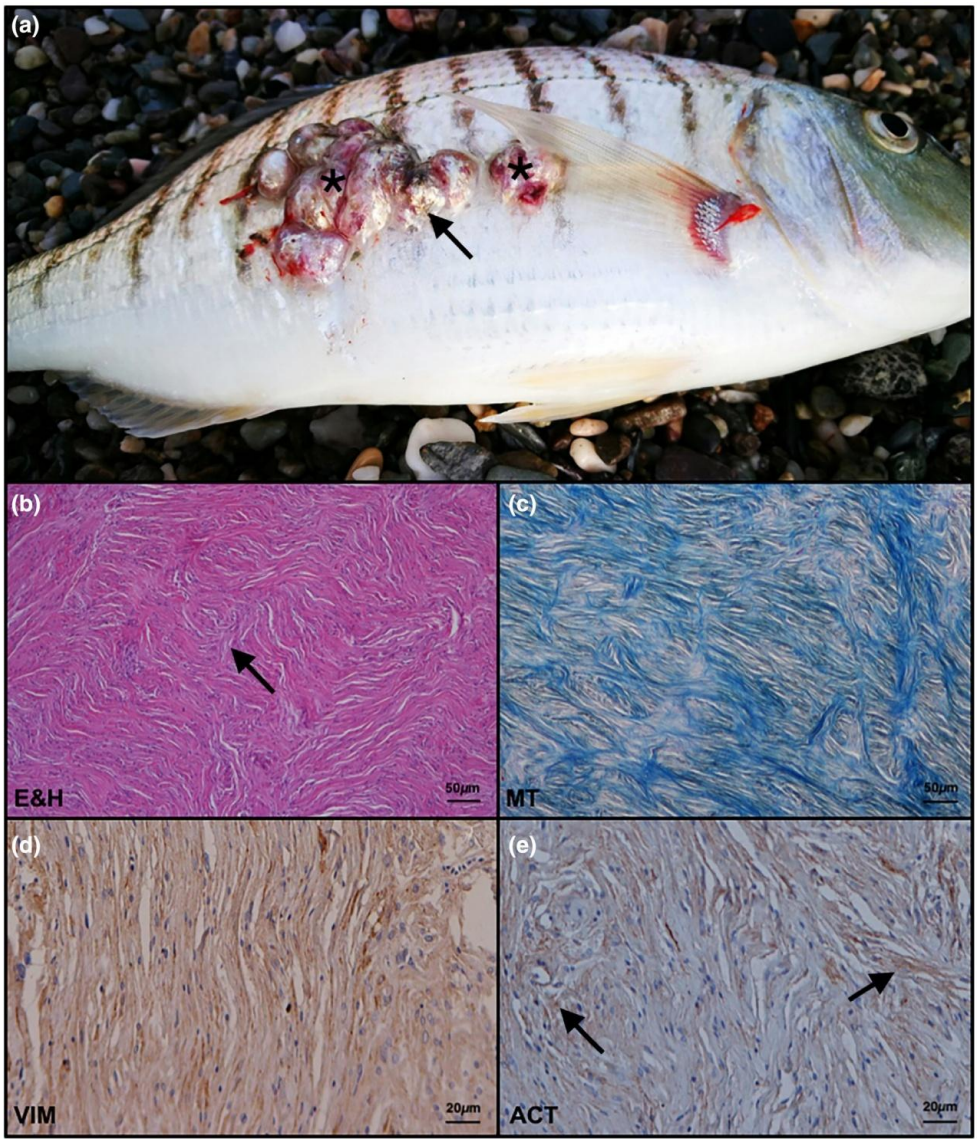

Fig. 3. Dermal fibroma in a Sand Steenbras: (a) multi-centric round mass (arrow), extending on right side of the fish, was smooth on the surface and white-gray, with diffuse hemorrhages (asterisks); (b) hematoxylin and eosin (H&E) stained histological section, showing irregularly interspersed spindle cells (arrow), with scarce extracellular matrix (20×); (c) Masson's trichrome (MT) stained section, confirming the collagen-producing nature of tumor cells (20×); (d) diffuse immunohistochemical positivity for vimentin (VIM; 20×); and (e) scattered immunoreactivity for muscle actin (ACT; arrows; 20×)

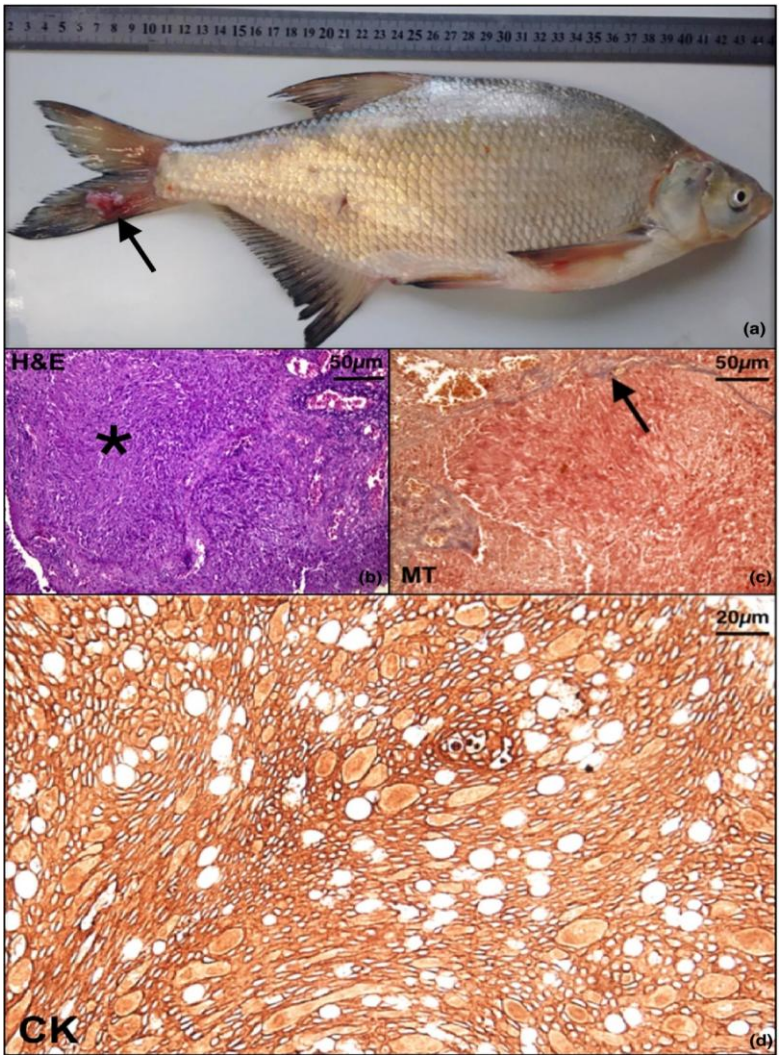

Fig. 4. Papilloma in a Bream: (a) irregularly round, white, firm mass on the right side of the caudal fin, growing outwards and deforming the fin; (b) (H&E) stained histological section, showing densely packed round to polygonal epithelial cells (asterisk; 20×); (c) Masson's trichrome (MT) stained section, showing a thin connective tissue bundle that provided a lobulated aspect (arrow; 20×); and (d) strong positive pan-cytokeratin (CK) reaction in the cell membrane of tumor cells (20×)

Cytokeratins

Many tumors are express cytokeratins, and their expression can help to identify the origin of a tumor. Cytokeratin is a useful marker for papillomas in fish and do intermediate filament proteins comprise one component of the cytoskeleton. These proteins are expressed in epithelial cells but are developmentally regulated [26].

Vimentin

"Is an intermediate filamental proteins and have been implicated in many types of tumors initiation and progression, including carcinogenesis, epithelial-to-mesenchymal transition, and the metastatic spread of cancer. This antibody is a sensitive and specific marker of mesenchymal derivation or differentiation" [27]. "Vimentin and smooth muscle antibodies are markers for mesenchymal tumors" [28,29].

Calretinin is a 29-kDa

"Calcium-binding protein; Calretinin is a specific marker used to distinguish schwannomas from neurofibromas in peripheral nerve sheath tumors (PNSTs) in humans [33] and has been reported in fish" [30].

S100 Proteins

"S100 are calcium-binding proteins present in both neuronal and nonneuronal tissues of higher vertebrates. S100 stains schwannomas, ependymomas, astrogliomas, almost all benign and malignant melanomas, and their metastases. S100 protein is also expressed in antigen-presenting cells, such as Langerhans cells in the skin and interdigitating reticulum cells in the paracortex of lymph nodes. S100 is a useful marker for schwannomas in fish" [31].

Melan-A

"Is a newly identified melanocyte differentiation antigen recognized by autologous cytotoxic T-lymphocytes (MART-1) and. is present in melanosomes and endoplasmic reticulum. Melan-A is exclusively expressed in the melanocytes of the skin and retina, in all melanocytic nevi, and in most primary and metastatic melanomas, as well as in some other tumors. The use of Melan-A antibody, unlike Masson–Fontana and Schmorl staining, was an essential tool in association with S100 antibody to differentiate melanocytic tumors from other pathological pigmentations" [34]. **"**It is a melanoma-associated protein used for melanoma discrimination" [32].

CD3 and CD79

CD3 and CD79 are used to differentiate T lymphocytes from B-lymphocytes in cases of lymphosarcoma [12].

Diagnosis of Tumor Cells (Tumor Markers Detection)

Immunoassays

"Immunoassays are bioanalytical methods in which the quantitation of the analyte depends on the reaction of an antigen (analyte) and an antibody. Immunoassays have been widely used in many important areas of pharmaceutical analysis such as diagnosis of diseases, therapeutic drug monitoring, clinical pharmacokinetic and bioequivalence studies in drug discovery and pharmaceutical industries" [35].

Immunohistochemistry

"A laboratory method that uses antibodies to check for certain antigens (markers) in a sample of tissue. The antibodies are usually linked to an enzyme or a fluorescent dye. After the antibodies bind to the antigen in the tissue sample, the enzyme or dye is activated, and the antigen can then be seen under a microscope" [36].

PCR

"PCR is an increasing number of molecular techniques have been recently

introduced for diagnosis of tumors and for predicting their behavior. The two most important tools are: - polymerase chain reaction (PCR) used to differentiate between monoclonal (neoplastic) and polyclonal (reactive) proliferation" [4,37].

FISH technique

The composite FISH marker may be useful for determining risk for subsequent development of lymph node metastases in patients with cervical cancer. FISH (fluorescent in situ hybridization) that used for detecting translocation is a characteristic for many tumors. Neoplastic cells are less cohesive (sticking together) than others and so are shed into fluids or secretions.

NB: Molecular markers

"Single genetic markers and a composite marker, comprised of three fluorescence ***in-situ*** hybridization (FISH) probes targeting the genes ***LAMP3***, ***PROX1***, and ***PRKAA1***, in pretreatment cervical biopsies from 16 lymph node positive cases and 15 lymph node negative controls from women with stage IB and IIA cervical cancers. As of yet, no molecular markers predicting lymph node metastases have been identified" [4,7].

HPLC

HPLC is a technique in analytical chemistry used to separate, identify, and quantify each component in a mixture. It relies on pumps to pass a pressurized liquid solvent containing the sample mixture through a column filled with a solid adsorbent material. Each component in the sample interacts slightly differently with the adsorbent material, causing different flow rates for the different components and leading to the separation of the components as they flow out of the column. HPLC has been used for manufacturing (e.g., during the production process of pharmaceutical and biological products), legal (e.g., detecting performance enhancement drugs in urine), research (e.g., separating the components of a complex biological sample, or of similar synthetic chemicals from each other), and medical (e.g., detecting vitamin D levels in blood serum) purposes [38].

Histopathology Technique (Exopholiated Pathology)

The shed cells (exfoliated) are evaluated for anaplasia (Exofoliative cytology) .Definitive diagnosis is made by observing the abnormal cells using histopathological methods. Histologic features of tumors are extremely useful in determining the differences between the benign or malignant tumors, if it still locally or metastatic spreaded. Whether or not it has been completely removed during a surgical biopsy, and what the overall prognosis is for the host.

Ultrastructural Abnormalities Detection

"The identification of tumor type is important for understanding the prognosis and for relating a specific tumor to possible environmental toxic or biological etiology. For these reasons, although ultramicroscopy and molecular biology have become necessary for diagnosis, especially in certain tumors, Immunohistochemistry remains a viable tool with which to easily discriminate cell origin. In particular, antibodies could be specific markers for several tumors" [39,40]. "In some gonadal tumors, the presence of a mass can be confirmed with ultrasound. Biopsy of tissue may not offer a clear diagnosis. Laparotomy of affected fish often reveals a circumscribed mass of gonadal tissue. Fish that are not excessively debilitated are excellent surgical candidates for removal of the mass" [12]. "Definitive diagnosis is made by observing the abnormal cells using histopathological methods" [7].

Immunofluorescence (IF)

"IF is an important immunochemical technique that allows for detection and localization of a wide variety of antigens in different types of tissues of various cell preparations. IF allows for excellent sensitivity and amplification of signal in comparison to immunohistochemistry, employing various microscopy techniques. There are two methods available, depending on the scope of the experiment or the specific antibodies in use: direct (primary) or indirect (secondary). Here, we describe preparation of specimens preserved in different types of media and step-by-step methods for both direct and indirect immunofluorescence staining" **(Kyueseok et al. 2019)**. "Earlier there were two major tools for estimating tumor markers; Enzyme-Linked Immuno Sorbent Assay (ELISA) and Radioimmunoassay (RIA)**"** [23]. "Immunohistochemical markers (Estrogen and progesterone receptors, ER, and PR), molecular tools (TMPRSS2: ERG fusion genes, gene expression profiles) as well as proteomic tools are now employed to quantify cancer markers. Some of these markers have been accepted for use in clinical practice (e.g., ER and PR, gene expression profiling in breast cancer). Many more are likely to be introduced into the market in the near future" [41].

Treatment of neoplasms: An example for Tilapia

Treatment of neoplastic disease in fishes that is generally restricted to surgical methods. Most cancers and tumors found in fishes have no cure or treatment. Internal tumors or cancers are also not diagnosed until the advanced stages of the disease, and when it is identified early, the position and placement of the tumor often makes it inoperable. This is the main reason most fishes with tumors and cancers are terminated (euthanized). However, there are some tumors which are treatable. For instance, gill tumor, which is caused by a thyroid problem, can be treated by placing the fish in water medicated with iodine.

-"Tilapia became one of the most important farmed freshwater species, being produced in more than 125 countries around the globe. Although genome assemblies have been available since 2011, most of the tilapia industry still depends on classical selection techniques using mass spawning or pedigree information to select for growth traits with reported genetic gains of up to 20% per generation" [42]. "Nile tilapia, *O. niloticus*, is one of the commercially important, fast-growing and well-adapted freshwater fish that produced intensively all over the world" [43]. "In Egypt, Tilapia is considered as the main cultured fish species and contributes with about 65.15% of the Egyptian fish production" [44]. "However, in recent years the tilapia culture, especially during summer, has faced higher mortality rates" [45]. "Tilapia (*O. niloticus*) and common carp (*Cyprinus carpio*) are both major farmed fish species globally. The two species might present a good model to study the genetic control of distribution of intermuscular bones in fish and its relation to bone fish tumors. Bone morphogenetic protein 4 (BMP4) gene is associated with tissue ossification and bone regeneration in mammals, but in fish its role in ossification remains understudied" [46-48].

CONCLUSION

Lastly, we can concluded that the following points considered a way to understand tumors as general and helps in its diagnosis and be easy in the future to detect the selective therapeutic measures for treatment patients and animals as well as fish :-

1- Tumour markers are commonly proteins associated with malignancy,
2- A tumour marker can be detected in a solid tumour, in circulating tumour cells in peripheral blood, in lymph nodes, in bone marrow, or in other body fluids (urine or stool)
3- A tumour marker can be used for population screening and for detection, diagnosis, staging, prognosis, or follow up of malignant diseases
4- Unspecific markers include the oncofetal proteins (such as the carcinoembryonic antigen or α fetoprotein) expressed by many different types of cancer

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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