Blue Biotechnology

REVIEW

Open Access

Beyond the petri dish: fish cell lines pioneering advances in biotechnology, genetic engineering, toxicity and disease solutions

Malik Wasim Mushtaq[1*], Irfan Ahmad Bhat[1], Mohd Ashraf Rather[1], Irfan Ahmad Khan[1], Raja Aadil Hussain Bhat[2] and Gowhar Iqbal[3]

Abstract

Adoption of cell culture technologies presents chances to address problems related to traditional aquaculture methods, such as disease outbreaks, habitat degradation, and overfishing. Cell culture systems provide fine control over growth conditions, reducing the amount of resources used and waste generated. Understanding cell properties, including species origin, cell type, and culture status, is crucial for effective and safe cell culturing. Zebrafish and medaka, as model organisms, offer advantages such as high fecundity, transparent embryos, and rapid maturity, making them ideal for genetic studies. Since the RTG-2 cell line from rainbow trout was established in 1962, fish cell lines from various tissues have been developed for research in virology, toxicology, and other biomedical fields. Characterization techniques include RAPD, microsatellite DNA profiling, and mitochondrial rRNA analysis. Fish cell lines are pivotal in viral disease research, toxicology, and intracellular pathogen studies. However, comprehensive characterization and genetic engineering of these lines remain limited. Advances in cell immortalization using telomerase and viral oncogenes enable continuous proliferation and genetic stability. Established cell lines from tissues such as skin, gill, muscle, heart, liver, and kidney have diverse research applications. Notable uses include studying viral diseases in salmonids, cellular processes in gill cells, and chemical cytotoxicity. Further development and characterization of fish cell lines will advance vertebrate biology and biomedical research.

Keywords Cell line, Immortalization, Genetic engineering, Transgenic research

Introduction

Fish cell lines have become valuable tools in transgenic, reproductive, toxicological, drug development, genetic environmental and virology research in aquaculture. These cell lines are established 700 cell lines from various fish species (for example: *Trichogaster lalius* (Dwarf gourami), *Paralichthys olivaceus* (Japanese flounder), *Geophagus Proximus* and *Astyanax bimaculatus*) and tissues, offering a platform for genetic manipulation and transgene expression [48, 71]. It offers certain advantage compared to mammalian cell culture such as wide temperature tolerance as well as hypoxia condition, similarity of genome to human (zebrafish ~ 80%) make an ideal to use for interdisciplinary areas [52]. Cells are isolated from various tissues of fishes based on that cell line produced such as embryonic stem cell line and germ cell line (primordial germ cells, gonadal germ stem cells) [65].

A thorough understanding of the inherent characteristics of cells is crucial for thriving and secure culture.

*Correspondence:
Malik Wasim Mushtaq
malikhamza13@gmail.com
[1] Division of Fish Genetics and Biotechnology, Sher-E-Kashmir University of Agricultural Science and Technology, Srinagar, India
[2] ICAR- Directorate of Coldwater Fisheries Research, Bhimtal, Uttarakhand, India
[3] Division of Fish Genetics and Biotechnology, Central Institute of Fisheries Education (CIFE), Mumbai, India

Regarding the threats associated with manipulating animal cells in cultures, three specific intrinsic properties have to be taken into account while conducting risk assessments. It is first necessary to consider the species of origin. Pathogens typically have specific species barriers, so cell cultures genetically closer to humans pose a higher risk to human health. Human or primate cells generally have a higher likelihood of harboring harmful organisms contrasted with non-human origins of cells [25]. Second, the cell type or tissue origin of cell lines should be contemplated. Types of cells have widely different within living lifespans: intestinal and certain white blood cells live a couple of days, red blood corpuscles in humans for about hundred days, and cells of liver usually don't demise. When it comes to grown-ups, brain cells are lost slowly with little to no replacement. This disparity affects the availability of certain cell lines. The state of the culture is another inherent aspect to take into account. The manipulation of primary cultures, cell lines, and continuous cell lines produced from primary cultures are all part of the diagnostic and research process. Typically, the most realistic in vitro portrayal of normal cellular responses found in vivo is provided by cell strains and primary cell cultures that are derived directly from organs or tissues. However, the amount of time available for characterising and detecting contaminating substances is restricted because of their limited lifetime [108].

Although *Oryzias latipes* (medaka) and *Danio rerio* (zebrafish) are considered early vertebrates, they offer numerous benefits compared to other animal models. They are highly fecund, with ovulation easily controlled by light, and they spawn frequently throughout the year. fertilised eggs injected microscopically is straightforward and cost-effective. Transparency is shown by embryos, enabling non-invasive monitoring of expression of gene dynamics within different in vivo tissues and organs, eliminating the need of offering experimental participants lives. Having between 20 and 40 percent larger genomes than mammals, they are uniquely suitable for large-scale mutagenesis studies among vertebrates. They reach sexual maturity in just 2 to 3 months, significantly expediting the generation of transgenic lines. Additionally, these model fish have well-established methods from genetics and molecular biology, such as knockdown, knock-out, and knock-in, making them excellent systems for studying vertebrate-specific biology in vivo [92]. Almost half of all known vertebrate species are fish and this provides a big opportunity to improve the development of models for cells and tissues used in biomedical sciences [85]. Cell lines used commonly are usually obtained from cancerous tumors, spontaneous transformation, or oncogenic cell immortalization. These alterations result in the establishment of continuously proliferating cell lines [46]. The RTG-2, originating out of the gonads of *Salmo gairdneri* (rainbow trout) was a groundbreaking achievement by Wolf and Quimby in 1962 marking the initiation of permanent fish cell lines. Following this milestone it became possible to effectively create many more fish cell lines. In 1980, Wolf and Mann conducted the first comprehensive review covering all aspects of fish cell and tissue culture, providing valuable insights into this field [106].

Scientists have established many cell lines of fish by extracting cells out of different bodily tissues aiming to detect and isolate piscine viruses. These cell cultures originating out of various species and tissues play a crucial role in investigating how different species respond to viral infections on a cellular scale. It is known that several viruses target specific organs or tissues emphasizing the need to create additional cell lines derived from diverse tissues and organs within a host organism. This is vital for accurately observing and studying infectious viruses [85].

Obtaining cell lines from various sources often comes with absence of confirmation or record-keeping regarding their passage number or condition. This oversight increases chances of working with inferior or malfunctioning cultures, potentially producing outcomes that are not reliable nor repeatable [144]. Microsatellite DNA identification [109] and random amplified polymorphic DNA (RAPD) have been used as techniques for characterising fish cell lines [99]. Analysis of mitochondrial rRNAs 18S and 16S sequences has also been employed [2], proving helpful in determining several fish cell lines. These are not only valuable in studying fish diseases but also play significant roles in broader areas such as carcinogenesis, toxicity, and expression and control of genetics, as well as replication and repair of DNA. Their applications extend beyond the realm of fish-related issues, contributing crucial insights in various scientific studies [11, 21, 63]. The cell lines can be transfected and holds promise as an effective tool for genetic manipulation, enabling the exploration of host–pathogen interactions [49]. Cell-based aquaculture systems utilizing cell cultures have the potential to revolutionize seafood production, enabling the creation of aqua food from various species to meet the growing demand of the expanding global population [54, 115].

Over time, it has proven empirically possible to extract a small number of everlasting fish cell lines from different fish species. These cell lines are essential for the growth of viral infections, the detection of viral illnesses, and the use of these models as in vitro research models in scientific studies [35, 85]. Nonetheless, little attention has been paid to thoroughly characterizing these cell lines and developing genetic engineering techniques for them.

This is despite the challenges associated with obtaining and maintaining models of genetically altered fish, other than zebrafish, because of back crosses, long generation cycles, size, and unique husbandry requirements. Additionally, due to their evolutionary history of two whole genome duplication events, salmonid fish present more challenges when it comes to genome alteration [35].

Establishment of different cell lines

The majority of fish cell lines come from healthy tissues such the skin, gills, liver, heart, spleen, kidney, brain and swim bladder. Fins and embryos are particularly common sources for these tissues used in primary cultures. After ovaries, fins are the 2nd most prevailing tissue employed in cultivation due to their notable self-renewal capability [47].It is notable that there are few cell lines derived from gonadal or ovarian tissues., despite the expected high mitotic activity in these areas. Prominent instances of cell lines derived from fish species include the ovary [82], fin and skin [83], spine [111], scale [4] and various other sources.

Fish cell lines have been studied using various methods. These include RAPD techniques [99], microsatellite DNA profiling [109] and mitochondrial rRNA sequence analysis [2]. A simpler proteomic method uses protein expression signatures with 2D gel electrophoresis and image analysis to identify and differentiate cell lines [137].

Immortalization of cells

The limited lifetime of normal somatic cells culminates in senescence following a consistent amount of divisions inside cells [58]. This ageing phenomenon stems from a pair of intertwined pathways: one prompts arrest of the cell cycle, controlled by tumour suppressor pathways such p16INK4a/Rb and p19ARF/p53 [79], while other involves crucial shortening of telomeres due to the end-replication dilemma during chromosome replication [10]. Spontaneous immortalization of a small subset of cells has been noted, typically associated with genetic alterations. A common modification seen in immortalised cells is the absence of functioning p53 or Rb proteins, which control important cell cycle checkpoints [18]. Moreover, various virus oncogenes like simian virus-40, adenovirus E1A and E1B possess the ability to immortalize cells across species [74].

Telomerase ribonucleoprotein's a catalytic component (TERT) exhibiting reverse transcriptase activity plays a pivotal role in synthesizing and maintaining telomeres, thereby aiding cells in evading reproductive senescence brought on by telomere diminution [18]. The favoured approach for cell immortalization involves the expression of TERT, especially in cells significantly impacted by telomere length, such as human cells [132]. Examination of several cell lines immortalised using telomerase has confirmed their maintenance of a genotype stability and the preservation of important phenotypic markers. The hTERT cDNA-containing eukaryotic expression plasmid is readily accessible in American Type Culture Collection (ATCC), facilitating research scholars in the immortalization of their own cells. Cell immortalization techniques have garnered considerable interest due to the resulting cell clones exhibiting persistent viability, excellent revival post- simplicity of storage and accessibility of management in culture. The aforementioned methods have primarily been utilized in generating everlasting human cell lines and other mammals. However, Barker et al. [12] first cataloged the constitutively high levels of long-term leukocyte lines from channel catfish with telomerase activity. Additionally, in 2002, Shau-Chi received a US patent (US 6,436,702 B1) for an immortal cell line produced from grouper (Epinephelus coioides). Notably, this legal claim outlined the transformation observation process, characterized by changes in the distribution of chromosomal numbers, plating efficiency, and FBS needs, and induction of endless cell division without external methods [85]. Various fish cell lines with their tissue of origin and characterization are given in Table 1 as under:

Applications

Cell lines play a crucial role in biological and medical research, serving as reliable and reproducible models for studying various cellular processes. Their adaptability has made them essential in numerous fields, including drug development and vaccine research. The key applications of cell lines are mentioned in Fig. 1 and described in detail below.

Fish cell lines applications in genetic engineering and gene-editing technology

Genetically modified fish cell lines hold significant potential for various biotechnological and clinical purposes. The introduction of CRISPR-Cas9 technology has transformed the field of genome editing [67, 68]. By harnessing CRISPR-Cas9, it becomes possible to generate enhanced fish cell lines, which in turn could greatly aid aquaculture biotechnological research, particularly in the realm of studying fish diseases. Utilizing genome editing technology to genetically modify cell lines could greatly increase fish cell lines' transfection efficiency, enabling their efficient utilization in virus production for vaccine development. Although the majority of applications for this approach have included gene editing in mammalian cell lines, fish cell lines are still in their early phases of application due to low transfection efficiency [39]. Knockout cells or animals are generated when CRISPR/Cas9 is co-expressed with a gRNA specific to the target

Table 1 Overview of notable fish cell lines

S.No	Designation	Species	Tissue	Characterization	Reference
01	RBTE 45	*Oncorhynchus mykiss*	Embryo	Not mentioned	Ristov and De Avila [113]
02	RT-gill W1	*O. mykiss*	Gill	Contaminated with mycoplasma, but eradicated	Bols et al. [20]
03	MG-3	*Cirrhinus mrigala*	Gill	Profile of three isoenzymes	Sathe et al. [117]
04	RG-1	*Labeo rohita*	Gill	Profile of three isoenzymes	Sathe et al. [116]
05	PSP	*Puntius schwanfeldi*	Fin	Not mentioned	Karunasagr et al. [73]
06	RTS11	*O. mykiss*	Spleen	Not mentioned	Ganassin and Bols [50]
07	GAKS	*Carassius auratus*	Scales	Secreted endothelin and had alkaline phosphate activity	Akimoto et al. [4]
08	SHHT	*Channa striatus*	Heart	Not mentioned	Zhao et al. [159]
09	TP-1	*Tor putitora*	Fry	Cell cycle analysis	Lakra et al. [84]
10	SICE	*Catla catla*	Eye tissue	12S rRNA sequence analysis using pEGFP vector transfection	Ahmed et al. [1]
11	PSF	*Etroplus suratensis*	Caudal fin	16sS rRNA and COI sequencing	Swaminathan et al. [131]
12	mRTP1B	*O. mykiss*	Pituitary gland	Expressed other pituitary-specific genes	Chen et al. [31]
13	CoE 35	*Onchorhynchus kisutch*	Embryo	Not mentioned	Ristow and De Avila [113]
14	SHK-1	*Salmo salar*	Head kidney	Not mentioned	Dannevig et al. [36]
15	PG-9307	*Paralichthys olivaceus*	Gill	Not mentioned	Tong et al. [134]
16	SAF-1	*Sparus aurata*	Fin	Not mentioned	Bejar et al. [16]
17	GF-1	*Epinephelus coioides*	Fin	Not mentioned	Chi et al. [34]
18	ASK	*Salmo trutta*	Head kidney	Not mentioned	Devold et al. [41]
19	TO	*S. salar*	Head kidney	Not mentioned	Wergeland and Jakobsen [145]
20	LJES1	*Lateolabrax japonicus*	Embryo	The cells differentiated into several types of cells after their treatment with every form of trans retinoic acid	Chen et al. [33]
21	WSF	*Acipenser transmontanus*	Fin	Sequence analysis of 16S rRNA	Wang et al. [138]
22	RGF	*S. salar*	Gill	Responded to human fibronectin & type 1 collagen using monoclonal antibodies	Butler and Nowak [28]
23	VSa13	*Sparus aurata*	Vertebra	Examining the expression and control of genes unique to bone and cartilage	Pombinho et al. [111]
24	HEW	*Melanogrammus aeglefinus*	Embryo	The sequencing of 2 genes related to housekeeping	Bryson et al. [26]
25	CSEC	*Cynoglossus semilaevis*	Heart	Expressing the GFP reporter gene transfection	Sha et al. [125]
26	Cod ESC	*Gadus morhua*	Embryo	Expression of a transcription factor of class V POU, known as ac-Pou2	Holen et al. [64]
27	TK	*Scophthalmus maximus*	Kidney	Transfected with pEGFP-N3 vector	Wang et al. [139]
28	SISE	*Lates calcarifer*	Blastula	Transfection defined by proliferate marker (BrDU) and CFLSM with pEGFP-N1	Parameswaran et al. [108]
29	SBB-W1	*Dicentrarchus labrax*	Brain	Using glial and neuronal markers for the immunostaining	Servili et al. [124]
30	LRM	*L. rohita*	Muscle	Invitro research	Yaswanth et al. [154]
31	SIMH	*Chanos chanos*	Heart	Transfected with pEGFP-N1 immunocytochemistry	Parameswaran et al. [107]
32	LRM	*L. rohita*	Trunk muscle	Invitro research	Goswami et al. [53]
33	CMM	*Clarius magur*	Muscle	Invitro research	Dhivyakumari et al. [42]

gene. Gene knockouts reveal a gene's function by changing the expression of the gene in a cell [67, 68].

Over the last several years, many genetic methodologies were explored and used to enhance aquatic species growth rate. The utilization of tools for genetic editing such as CRISPR/Cas9, TALENs, and transgenisis has contributed to expanding our understanding of somatic growth regulation in fish Zhong et al. [160]. Zebrafish might be subjected to in vivo CRISPR/Cas genome editing by introducing site-specific insertion/deletion (indel) alterations utilising this technology [66]. Researchers have conducted investigations on various fish species to genetically modify genes for diverse objectives. Researchers have gained greater knowledge on somatic growth

Fig. 1 Different application of fish cell lines

modulation in fish because of the use of genetic editing technologies including CRISPR/Cas9 and TALENs [13, 133]. A number of researchers have worked on fish species to edit the genes for different purposes.

While the CRISPR/Cas9 technology introduced a revolution in molecular biology, providing a specific and efficient tool for inducing certain mutations in living cells, since its discovery, various gene-editing strategies with the use of this systemin cell lines of mammals have been widely applied. In contrast, relatively limited knowledge is available concerning genetic manipulation of fish cell lines. Recently, genome editing technology has been applied to knockout myostatin in numerous fish species, spanning from model to marine fish, as evidenced by studies conducted by (Chang et al. [30]; [23] and Jeong et al. 2019). Dehler et al. [39] develop a productive technique for gene editing using CRISPR-Cas9 in a fish cell culture. Wu et al. [151] uses this technology to knockout mstnb gene in *Oreochromis niloticus* (Nile tilapia). Liu et al. [93] had utilized the CRISPR-Cas9 system technique for genetic alteration in medaka fish cells with a pre-assembled ribonucleoprotein combination of gRNA and Cas9.

Together with Kyoto University and Kinki University, Regional Fish Co., Ltd. has been working in cooperation with the Japanese Ministry of Agriculture, Forestry, and Fisheries, as well as the Ministry of Health, Labour, and Welfare on editing genomes for "Madai" red sea bream. Using the gene editing method of CRISPR, knockout of the gene that inhibits muscle growth was realized to show an increase in skeletal muscle mass up to 17% in genome-edited fish versus conventional fish. This produced an edible part of the fish that was 1.2–1.6 times larger than conventional varieties, along with an impressive feed utilization efficiency improvement of around 14% over conventional varieties Kishimoto et al. [78]. Various applications of Fish cell lines in genetic engineering and gene editing technology are given in Fig. 2 as below:

Uses of fish cell lines in transgenic research

During the past few years, there has been a need for the development of transgenic fish with improved various commercial traits of growth, meat quality, and disease resistance in order to meet the global demand by drastically raising the output and utilization of cell lines for transgenic studies. Fish Cell lines has played a very key role to study the basic fish biology and molecular biomarker development. Indeed, Wang et al. [142] established with success that techniques such as recombinant baculovirus delivery have been used in transducing fish cells. Among these are the *Mylopharyngodon piceus* bladder/fin/kidney; spermatogonia of *O. latipes*, SG3; and the embryonic fibroblast, ZF4, cells of Danio rerio that have helped in the generation of stable transgenic cell lines. Furthermore, bulk foreign gene transfer into fish eggs and sperm has been investigated using methods like electroporation, and three main steps include the introduction, expression, and germ line transmission

Fig. 2 Application of genetic engineering and gene-editing technology

of transgenes, allowing production of transgenic fish expressing foreign genes. Permanent fibroblast cell lines, which originated from fish muscle, have already been used in experiments on genetic manipulations and transgenics; the cells were utilized to confirm the possibility of growth with genetic stability. All these methods prove that we can use fish cell lines can contribute to the promotion of studies on transgenics in aquaculture [14].

Applicaton in drug development

The fish cell line in pharmaceutical/drug research becomes attractive to researchers and pharmaceutical industries following challenges associated with the traditional animal testing methods. Whereas cell line allows easy transportability, low maintenance cost, the ability to mimic the certain disease condition, not required to sacrifice the animal, rapid growth rate, higher susceptibility to bacteria, virus infection, transgene expression, reproducible results, and also provides drug-drug interactions for a combination effect of drugs [70]. For the purpose of determining cytotoxicity of putative medications and to perform high throughput screening of possible compounds, cell-based assays have become an essential component of the pharmaceutical industry. Dose optimization, drug transport, pharmacological analysis, cellular targeting, security, pharmacology, and quality control are further relevant applications [5]. Fish cell cultures may be useful in the discovery of therapeutic targets such receptors as well as in the study and development of medications intended to assist fishAntiviral drugs are often screened using fish cell lines. Acyclovir, a popular antiviral used to treat human herpesvirus infection, proven to be successful in treating channel catfish viral disease in Chinook salmon embryo cells, according to research by Hao K. et al. [56]. Additionally, it was shown that acyclovir had potent antiviral action to prevent the infection of cyprinid herpesvirus-3 (CyHV-3) in Koi Fin cells and Common Carp Brain (CCB). In CCB cell lines, exopolysaccharides extracted from the algae Arthrospira platensis reduced KHV multiplication..

Researchers developed a cell line, the *T. lalius* caudal fin cell line, for studies on various viruses such as the spring viraemia virus, hirame rhabdovirus, infected spleen and kidney necrotic virus and red sea bream iridovirus. They reported that FCL is persistent to the Infectious Spleen and Kidney Necrosis Viru (ISKNV) infection and useful in understanding ISKNV pathogenesis in fishes. Infection of infectious haematopoietic necrosis virus (IHNV), viral haemorrhagic septicaemia virus (VHSV), infectious pancreatic necrosis virus (IPNV), spring viremia of carp virus, channel catfish virus, or grass carp reovirus in the heart derived gold fish cell lines has proved that GH cell line is a wonderful tool for viral pathogenesis studies [72]. Studied FCL in drug development are shown in Table 2.

Use of cell lines in fish health management

Fish cell lines represent an essential tool for the study of a huge number of critical themes involving fish development, illness, biotechnology, genetics, and reproduction. Furthermore, these cell lines have been proved to be very good in vitro models for the investigation of immunology, pathology, and toxicity. Some commonly used cell lines obtained from fish cells are given in Table 3 as below:

The most frequent application for fish cell lines is in characterising and isolating viruses. Fish cell lines used in diagnosis and identification of intracellular fish infections include, RTG-2, EPC, FHM, BF-2, CHSE-214, EPC, and CCO [7]. The economic loss due to fish disease and the extensive utilization of many medicines and additional substances posing a serious threat to the aquatic environment have placed it among the most important issues for sustainable aquaculture production [104]. Several cultures were employed to investigate the pathogenesis and development of parasites. The adhesion and transformation of several stages of the fish ectoparasite *Ichthyophthirius multifiliis* were facilitated by the EPC cell line. The non-specific reaction of EPC to destroy *Gyrodactylus derjavini*, the fish parasite was examined by Buchmann et al. [27]. *Loma salmonae*, a microsporidian parasite, was studied for its phagocytic activity using primary cell cultures obtained from salmonid fish. The comparative growth of two microsporidians infecting salmonid fish and AIDS patients was studied using primary cultures of rainbow trout kidney [40].

Fish cell culture has a huge potential for developing techniques and tools for aquaculture disease management. Sectioning of the interactions of pathogens with their hosts by in vitro models using cell culture methods and laboratory models aids in the in-depth understanding of the intricate interactions which govern pathogenesis and disease development [57]. Yashwanth et al. [153] developed a novel *Amphiprion ocellaris* ornamental fish cell line and study susceptibility to nervous necrosis virus. Fish cell lines could, therefore, find application in studies of the aetiology of the disease, development of diagnostic tools, and drugs and vaccines to control fish diseases.

Table 3 Examples of widely used fish cell cultures [150], Lannan et al. [86]; [128]

S. No	Cell line	Fish and tissue of origin
1	RTG—2	O. mykiss-gonad
2	CHSE-214	*O. tshawytscha*-embryo
3	FHM	*Pimephales promelas*- caudal peduncle
4	BB	Brown bullhaead—caudal peduncle
5	CAR	Gold fish fin
6	EPC	*Epithelioma papulosum cyprini*
7	SSN-1	Snakehead fin
8	BF-2	Bluegill fry—caudal peduncle
9	E11	Snakehead fin
10	SBK-2	Seabass kidney
11	KO-6	kokanee salmon ovary
12	CHH-I	chum salmon heart
13	RTH-149	rainbow trout hepatoma

Uses of fish cell lines in vaccine development

A dependable and durable substitute for current vaccine production methods is cell-culture-based technology. A significant number of live fish are needed for the vaccine's development and potency tests. When producing and evaluating the effectiveness of fish vaccines, fish cell

Table 2 Fish cell lines in drug development

Cell line name	Viral/ bacterial assessed	Outcome of the study	References
Carassius auratus (Grass gold fish)	Transfection using lipofectamine LTX and Xfect	This cell line useful for artificial fish meat production and myogenesis related functional genes studies	(Li et al. [89])
Paralichthys olivaceus (Japanese Flounder)	Bohle virus (BIV), Lymphocystis disease virus (LCDV), Hirame rhabdovirus (HIRRV), Viral hemorrhagic septicaemia virus (VHSV), and infectious haematopoietic necrosis virus (IHNV)	This FCL will help to study immune gene and mechanism of fish for disease prevention as well as treatment	(Yucong et al. [155])
CPB *Siniperca chuatsi* (Chinese perch)	Infectious spleen and kidney necrosis virus (ISKNV)	The cells showed high susceptibility to ISKNV, with a viral titre of 6.58–6.62 log TCID50 ml − 1. The CPB cell line was demonstrated to be a useful in vitro tool for ISKNV propagation and gene expression studies	(Fu et al. [49])
AFF *Pterophyllum scalare* (Angelfish Fin)	Serratia marcescens and Proteus hauseri, Cyprinid herpesvirus 2 and viral nervous necrosis virus (VNNV)	The AFF cell line has potential for future studies, including isolation of iridoviruses affecting angelfishes	(Swaminathan et al. [130])

culture might be used instead of entire living fish. Based on Wang et al. [141], the primary application of cell lines such as chicken embryo fibroblasts and Madin Darby canine kidney has been in the manufacture of viral vaccines. Compared to mammalian cell lines, transfection efficiency is lower in fish cell lines. Recombinant protein and other products cannot be produced from low transfected cell lines. The transfection efficacy of mammalian cell lines was increased to 100% with the help of the optimal kind of cell and procedure combination [88]. There are very few experiments being conducted utilizing fish cell lines to generate viral vaccines. Large-scale synthesis of vaccines, interferons, blood clotting factors, insulin, growth factors, lymphokines, interleukins, hormones, viruses, enzymes, and anticancer medicines is made possible by cell cultures [158]. When it comes to producing biologicals in large quantities, fish cell lines are more cost-effective than mammalian cell cultures. Fish cell cultures infected with genetically modified baculoviruses can function as tiny factories, expressing significant amounts of economically relevant proteins. Many FDA-approved therapeutic proteins are produced in human cell lines [43]. Fish cell cultures might be used for similar experiments.

The viruses that were grown in Grunt Fin cells were used to create the formalin-inactivated RSIV vaccine [51]. For iridovirus and NNV protection, a number of fish virus vaccines that are either inactivated or attenuated have been produced [103], and some of these have been brought to the commercialization level [118]. To produce vaccines against megalocytivirus, betanodavirus, herpesvirus, and aquareovirus, further work is needed to create specialized cell lines that promote the growth of these viruses, as there are currently few cell lines available for their replication. Modern vaccine technology has many uses for fish cell cultures, including recombinant and DNA/RNA particle vaccinations. While several are presently undergoing trials to create vaccinations, only several fish cell lines are being utilised in the viral propagation process that led to the invention of vaccines and diagnostics.

Uses of fish cell lines in cancer research

In order to investigate the mechanisms and roles of different carcinogenic chemicals, the process of causing cellular death, DNA methylation, histone changes, tumor suppressor gene expressions, etc., normal cells can be transformed by chemical exposure, radiation, and viruses into cancerous cells. In cancer biology, fish cell lines are used to investigate DNA repair activity, molecular damage, and the process of procarcinogen activation. Goldfish erythrophoromas, goldfish fibroblast cell lines, and fathead minnow cells were employed in research on the mechanism of activation of procarcinogens, and therefore, the destruction and repair of genetic components [55]. The development of cell culture systems for monitoring mutagenic and carcinogenic chemicals in aquatic environments has made significant progress [19, 96]. For instance, RTG-2 cells from rainbow trout and BF-2 cells from bluegill fry have been shown to respond to sediment extracts contaminated with aromatic/chlorinated hydrocarbons and heavy metals in a manner similar to their response to known mutagenic and carcinogenic compounds [80]. Additionally, Bailey and colleagues [110] utilized isolated hepatocytes from rainbow trout to study aflatoxin B1 metabolism and its interactions with DNA. They also developed a cost-effective and sensitive assay that can be applied to both carcinogenesis and teratogenesis using rainbow trout embryos [62].

An innovative system combining embryos with primary cell cultures was developed using *Cyprinodon variegatus* (sheepshead minnows), which has proven effective for assessing teratogenic and carcinogenic effects [97]. Further advancements include the cloning of a gene homologous to the c-myc proto-oncogene in rainbow trout, which was found to be transcriptionally active [135]. This breakthrough provides a DNA probe for screening fish tumors and transformed cell lines for chromosomal translocations, rearrangements, and other abnormalities associated with the myc oncogene in higher vertebrates. The presence of proto-oncogenes in lower vertebrates offers valuable insights into the evolution of these genes and enhances our understanding of cell proliferation and tumorigenesis.

This potential is further highlighted by discoveries of factors involved in mesoderm induction in amphibian embryos (Xenopus sp), which are related to mammalian transforming growth factor β [77, 114, 143]. Moreover, the reliance on mammalian sera containing platelet-derived growth factors as primary mitogens for culturing most teleost cell lines underscores the conserved nature of growth factors, their receptors, and the fundamental mechanisms that regulate cell growth across vertebrates [63].

Uses of fish cell lines in disease management

Viral diseases lead to considerable losses in farmed salmonid fish populations [110]. To find, separate, and investigate the viruses responsible for diseases, 9 cell lines were established from 5 salmonid species. These species and the cell lines that correspond to them are as follows: chinook salmon with the cell lines CHSE-II4 and CHSE-214; coho salmon with the cell line CSE-119; sockeye and kokanee (landlocked sockeye) salmon (*Oncorhynchus nerka*) with the cell lines SEE-5, SEE-30, and KO-6; chum salmon (*Oncorhynchus keta*) with the cell line CHH-I;

and steelhead (anadromous rainbow) and rainbow trout (*Salmo gairdneri*) with the cell lines STE-137 and RTH-149. These cell lines have been maintained in culture for periods ranging from 5 years (CHH-I) to 21 years (CHSE-II4, CSE-119, STE-137, and RTH-149). These comprise the first-ever cell lines from the genus "Oncorhynchus," all of which are generated from economically significant species and allow homologous cell cultures to be used to examine viruses in these priceless fish stocks [149]. These cell lines are extensively utilized in research on viral diseases affecting fish [3, 61, 76, 101, 146, 148].

Most physiological research to date has utilized either primary gill cultures, perfused gills, or whole organisms. There has been minimal use of gill cell lines for fundamental research thus far. But Ebner et al. [45] recently revealed using the RTgill-W1 cells to study the subcellular localisation and pattern of activation of the mitogen-activated protein kinase ERK. This research demonstrates the potential of gill cell lines for studying cellular and molecular processes in fish. Furthermore, Krumschnabel et al. [81] examined RTgill-W1 cells to understand their process of apoptosis. Their evaluation focused on the activation of effector caspases, nuclear condensation, variations in the potential of the mitochondrial membrane, and the reduction in cell volume associated with apoptosis.

Viruses are obligatory intracellular parasites that depend on the functions of host cells to replicate and spread. Because of their many functions in virology, including viral detection, identification, propagation, isolation, confirmation, and characterisation, cell cultures are referred to as "the gold standard" [69]. OIE (Office International des Epizooties) standards need cell cultures for viral illness detection and confirmation because of the importance of cells in virology. Animals can be effectively substituted by fish cell cultures, particularly in the field of virology. Compared to the unknowns surrounding the acquisition of viruses from diseased animals for scientific reasons, cell cultures can serve as dependable sources of viruses [147].While viruses exploit host cell mechanisms for reproduction as ultimate parasites, cell lines can also aid in studying obligatory intracellular parasites of bacteria and protozoa. For example, the RTgillW1 cell line could facilitate research on microsporidia that invade gills like "*Loma salmonae*" [75]. This pathogen is responsible for microsporidial gill disease in salmonids, a significant infectious ailment affecting Chinook salmon raised in aquaculture in Canada [129]. Gill cell lines offer valuable opportunities for investigating ectoparasites that infect gills. Noga [102] examined "*Amyloodinium ocellatum*," a dinoflagellate frequently present in the gills of fish, using the G1B cell line and assessed the effectiveness of an antiprotozoal medication in vitro. The RGE-2 cell line was created expressly to study amoebic gill illness brought on by "Neoparamoeba" species. It was derived from the gills of Atlantic salmon [28]. In contrast to other fish cell lines obtained from other tissues, Lee et al. [87] showed the strong development and high production of a laboratory strain of "*Neoparamoeba pemaquidensis*" utilising the RTgill-W1 cell line, underscoring its appropriateness and specificity for researching gill infections. Therefore, gill cell lines represent indispensable tools for exploring organ-specific pathogen interactions in fish gills.

Uses of fish cell lines in toxicity testing

Gill cell lines offer a robust platform for detailed investigations into the biotransformation and cytotoxicity of substances at the gill level, surpassing what can be achieved in vivo. Both FG-9307 and RTgill-W1 have been instrumental in assessing the toxicity of few substances. For example, FG-9307 cells were used to evaluate the toxicity of organophosphorous pesticides [90, 91], while RTgill-W1 has been employed in studies assessing the impact of wastewater from industry [38], including those from petroleum refineries [122]. RTgill-W1 has also been pivotal in evaluating the hazards posed by polycyclic aromatic hydrocarbons (PAHs) and metals such as copper, cadmium, zinc, iron, and nickel [37, 119–121]. Fish cell cultures serve as appropriate substitutes for animals and are widely utilized as laboratory models for environmental toxicology research, particularly cytotoxicity analysis, as they are pertinent examples of the aquatic environment. It is possible to assess the genotoxicity of compounds, metabolism, DNA binding, and mechanism of action in addition to avoiding exorbitant expenditures and inconsistent findings. According to Caminada et al. [29], fish hepatoma cell lines can be employed for assessment of the xenobiotic efflux activity of human medications. Recently, Bopp et al. [24] utilized RTgill-W1 cells to investigate copper toxicity, suggesting that copper-induced viability loss and gill genotoxicity in trout may be partly triggered by reactive oxygen species generation. Similarly, studies using RTgill-W1 have examined the toxicity of polybrominated diphenyl ethers (PBDEs) [126], revealing a potential mechanism involving oxidative stress in cell injury, findings corroborated by tests using the trout liver cell line RTL-W1. Extensive research has revealed that different inorganic and organic pollutants in aquatic environments significantly influence the vulnerability of farmed fish to diseases [6, 22, 44, 156, 157].

Scientists have employed cell culture methods to explore the mechanisms behind the immunomodulatory effects of heavy metals and fungal toxins, and to understand their impact on fish health [136]. For example,

Aflatoxin B1, a mycotoxin, exhibits carcinogenic and immunomodulatory effects in numerous vertebrates. Rainbow trout, in particular, are highly vulnerable to the carcinogenic effects of Aflatoxin B1 when exposed through their feed or water Sinnhuber et al. [127]. The mechanisms behind aflatoxin B1-induced hepatic carcinogenesis in trout have been explored in vitro using isolated hepatocyte cultures. These studies focus on the biotransformation of aflatoxin B1 by hepatic cytochrome P450 (CYP) enzymes, examining the expression of CYP and the presence of DNA mutations. Additionally, the immunomodulatory effects of aflatoxin B1 in rainbow trout have been investigated through in vitro assays with leukocytes from salmonids that were exposed to the toxin in feed or water during their embryonic stage [105]. Conversely, the toxicity of compounds like polycyclic aromatic hydrocarbons and aflatoxins is primarily due to the metabolites formed during the biotransformation of the original compounds. This process can be particularly studied in vitro using fish cell cultures to observe the effects and mechanisms involved [15, 94]. Furthermore, studies have shown that a rainbow trout liver cell line (RTL-W1) exhibits greater sensitivity to polycyclic aromatic hydrocarbons, as evidenced by higher expression levels of cytochrome P4501A, compared to isolated hepatocytes in primary culture [15]. As an alternative to traditional organotypic cultures, researchers have utilized co-cultures combining isolated rainbow trout hepatocytes with the RTG-2 cell line. This approach allows for more specific studies on the interactions and responses of these cells to environmental toxins such as polycyclic aromatic hydrocarbons [123].

Uses of fish cell lines in reproductive bitechnology

Sertoli cell lines and male germ cell lines serve as crucial resources for fundamental research, particularly in studying reproductive biology and exploring their applications in regenerative medicine contexts [140]. Sertoli cell lines provide a robust supply of male germ cells such as spermatocytes and spermatogonia, alongside Sertoli cells. This enables focused exploration into the intricate genetic and epigenetic mechanisms that regulate spermatogonial mitosis and spermatocyte meiosis. Researchers utilize these cell lines to delve into specific aspects of gene regulation, signaling pathways, and the roles of non-coding RNAs like microRNAs and lncRNAs, as well as DNA methylation. These studies aim to elucidate how these factors control the processes of differentiation and self-renewal in male germ cells, offering insights into reproductive biology and potential applications in regenerative medicine [152]. For instance, using the spermatogonial stem cell (SSC) line C18-4, researchers have demonstrated that Nodal is crucial for encouraging mouse SSC self-renewal through the Smad2/3 pathway and activation of the Pou5f1 gene [59]. Additionally, it has been demonstrated that Glial cell-line derived neurotrophic factor (GDNF) increases the survival and proliferation of mouse SSCs by triggering the transcription factor and the Ras/ERK1/2 signalling pathway [60].

Recently, a human SSC line has been used to demonstrate that miRNA-133b participates in regulating human SSCs and, more precisely, increases human Sertoli cell proliferation. These reports indicate the different molecular mechanisms involved in the regulation of spermatogonial and Sertoli cell functions, thus teaching us about reproductive biology and opening perspectives for applications in regenerative medicine. Sertoli cell lines, like SerW3 and 93RS2, are representative in vitro models to study toxicology in the male urogenital system and to screen the drug development process for treatment of testicular tumors.

Fish stem cells hold significant promise for various biotechnological applications, with particular advancements and interest in areas such as gene targeting, germ cell transplantation, and semi-cloning through nuclear transfer. Gene targeting, combined with mouse embryonic stem cells, forms the foundation of knockout technology. However, while GT events are highly desirable, they occur much less frequently than random integrations. A technique known as positive–negative selection, which relies on drug selection, significantly reduces random events and enhances homologous recombination in mouse ES cells [95].

In fish, conditions for gene transfer and drug selection in the MES1 cell line have been optimized to advance GT applications. It was demonstrated that the expression of selectable genes providing resistance to neomycin, hygromycin, or puromycin effectively conferred resistance to G418, hygromycin, or puromycin, respectively, for positive selection. Additionally, the expression of the herpes simplex virus thymidine kinase gene induced sensitivity to ganciclovir, enabling negative selection. These findings confirm that PNS is effective in MES1 cells as well [32].

Uses of fish cell lines in nutrition and metabolism

Various types of fish cell lines can be used to study the feed formulation, effects of various nutrients on the fish health, to study the digestion and asslimation in various commercially important fish species so fish cell line will act as model to study various process related to fish nutrition. Primary cultures of myoblasts, hepatocytes, and adipocytes have been extensively utilized to investigate the molecular mechanisms associated with fish nutrition [54].

In a study conducted by [100], they observed the role of the RTH-149 RT hepatoma-derived cell line to explore

questions related to nutrition. Their research concentrated on key pathways including macroautophagy (also known as autophagy), the mechanistic target of rapamycin pathway and the general control nonderepressible 2 pathway. These pathways are essential for regulating cellular homeostasis through amino acids and are central to understanding nutrient-sensing signaling mechanisms. Various fish cell lines have been employed as in vitro models to investigate the desaturation and elongation of various polyunsaturated fatty acids [17]. Uses the cell line as a models to study the the pro-inflammatory mechanisms that connect dietary PUFAs to cardiac lesions in salmon, while as [9] study how fatty acid diets influence inflammatory responses in fish.

Fish cell line in respect to cell-based aquaculture for sustainable aquaculture growth

Cellular agriculture involves the creation of agricultural items using cell cultures instead of whole organism. Problems in conventional animal husbandry, concerns pertaining to animal welfare, the environment, and public health, have increasingly been putting focus on this technology as a solution. A cellular aquaculture concept was first discussed by NASA as a research program for the production of goldfish cells containing edible muscle protein for extended space missions [115]. Improved comprehension of muscle cell and tissue culture myogenesis is needed to utilize the full potential of cellular aquaculture. Producing seafood from cultured fish tissues and cells, a concept that is still gaining ground, is also one plausible approach to help address the associated issues with marine capture and industrial aquaculture. Such is the case for biomedical engineering advances for instance, closed-system bioreactor technology that is producing cells from terrestrial animals, which could be utilized toward the scaling up of marine animal cell production for cell-based seafood. Development of muscle cell lines has come from marine as well as freshwater fish species [54]. Yaswanth et al. [154] has developed muscle cell line LRM from Labeo rohita for in vitro research. [53] developed a new muscle cell line from Labeo rohita named as LRM.

Efforts have been undertaken to develop crustacean tissues cell lines, aiming to enhance their isolation and maintenance methods. These efforts have involved utilizing short-term cultures derived from several isolations of primary cells, as documented by Rinkevich in 2005. While short-term cultures suffice for laboratory-scale investigations, they need to meet the demands of long-term objectives associated with mass production and commercialization in cell-based seafood applications. To achieve its full potential, the advancement of cell-based seafood research necessitates a deeper comprehension of culture of fish muscle cells and tissues. Further, exploration into tailored serum-free media formulations suitable for fish cell culture, and the development of bioreactor designs specifically optimized to meet the requirements of fish cells for industrial manufacturing. Studies focusing on the molecular mechanisms of cell-based systems will furnish essential research data for the production of fish through cellular methods. Certain inquiries into harvested muscle tissues from freshwater and marine fish offer intriguing perspectives on the feasibility of establishing a developing a muscle cell culture system [112]. Research needs to delve deeper into the biology of fish muscle cells, including their growth mechanisms, nutrient requirements, and cellular signaling pathways. Therefore, it is imperative to conduct research focusing on the sustained culture of these cells over extended periods to facilitate progress towards achieving these goals.

Future perespective of fish cell lines

The lack of appropriate fish cell cultures poses a significant challenge in isolating pathogens that are specific to particular species and tissues. Moreover, established fish cell lines are not readily accessible to research repositories, and the potential of fish as a source of cell lines remains largely untapped. For a fish cell line to be widely adopted as a reliable research tool, it is crucial to implement standardized media, reagents, and equipment, alongside rigorous quality control protocols. Additionally, proper characterization and thorough documentation of the cell lines developed are essential for their successful use in research. Using incorrectly identified or cross-contaminated cell lines can lead to invalid experimental results, making it essential to authenticate cell lines as part of the cell culture process. Cross-contamination has persisted due to improper handling and neglecting best practices in tissue culture. These issues raise concerns about the reliability of cell lines in biological research, as they may produce inconsistent, illogical, or non-reproducible results, potentially leading to unnecessary further investigations. To ensure cell lines are used effectively as models in research, they must be thoroughly characterized beforehand. Techniques such as DNA fingerprinting with multi-locus probes, short tandem repeat (STR) profiling, karyotyping, isoenzyme typing, and HLA typing can help identify and characterize cell cultures [98]. Various screening methods are also necessary to detect contamination, such as mycoplasma infections, which can adversely affect cell health over time without visible symptoms. Utilizing an appropriate cell model enhances the effectiveness and productivity of research. When selecting medium and reagents for culturing stem cells or primary cells, several factors need to

be considered. Tools like cryo-containers and proliferation assays are essential for maintaining cell health.

Conclusion

The fish cell lines have shown promising outcomes in a number of important areas of aquaculture in vitro research, such as nutrition and metabolism, virology, vaccine development, and fish production by transgenesis. In contemporary biological research, the usage of fish cell culture is growing. Research on fish cell culture, however, faces numerous difficulties, including contamination and misidentification. Fish cell lines will be used far more frequently to treat biotechnological interventions, fish disease and genetics as aquaculture expands globally.

The establishment of the RTG-2 cell line from rainbow trout gonadal cells in 1962 marked the beginning of extensive fish cell line development, crucial for research in virology, toxicology, and other biomedical fields. Characterization techniques, such as RAPD, microsatellite DNA profiling, and mitochondrial rRNA sequence analysis, are vital for ensuring the accuracy and reproducibility of cell lines. Despite their significance, many fish cell lines lack thorough characterization and genetic engineering, limiting their potential. Advances in cell immortalization, particularly through telomerase expression and viral oncogenes, have allowed for continuous proliferation and stable genetic properties. Fish cell lines obtained from various tissues, such as liver, gill, heart, skin, and kidney, have specific applications in studying viral infections, toxicology, carcinogenesis, genetic regulation, DNA replication, and repair. These cell lines also play a crucial role in understanding intracellular pathogens and ectoparasites. Notable examples include the RTgill-W1 cell line used to investigate mitogen-activated protein kinase activation and apoptotic mechanisms. Furthermore, fish cell lines offer robust platforms for studying cytotoxicity and biotransformation of chemicals, surpassing what can be achieved in vivo. For instance, the toxicological impact of industrial waste products has been assessed using RTgill-W1 cells. and various metals, contributing valuable insights into environmental toxicology. The continued development and thorough characterization of fish cell lines will enhance our understanding of vertebrate biology and support significant advancements in biomedical research. This will lead to more accurate in vitro models, facilitating the study of cellular mechanisms and improving the reliability of research outcomes in biomedical sciences [8].

Acknowledgements
Not Applicable

Authors' contributions
1. Malik Wasim Mushtaq: Conceptualization, Methodology, Writing – Original Draft. 2. Irfan Ahmad Bhat: Supervision, Formal Analysis, Visualization, Review & Editing. 3. Mohd Ashraf Rather: Data Curation and Supervision. 4. Irfan Ahmad Khan: Data Curation and Supervision. 5. Raja Aadil Hussain Bhat: Investigation, Validation, Review & Editing, Resources. 6. Gowhar Iqbal: Literature Review and Materials Provision.

Funding
No specific funding was received for the preparation of this review.

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable. This review paper did not involve any studies with human participants or animals performed by any of the authors.

Consent for publication
Not applicable. This manuscript does not include any individual person's data in any form.

Competing interests
The authors declare no competing interests.

Received: 14 November 2024 Accepted: 16 January 2025
Published online: 04 February 2025

References

1. Ahmed IVP, Chandra V, Parameswaran V, Venkatesan C, Shukla R, Bhonde RR, Hameed ASS. A new epithelial-like cell line from eye muscle of catla (Catla catla): development and characterization. J Fish Biol. 2008;72:2026–38.
2. Ahmed IVP, Chandra V, Sudhakaran R, Rajesh Kumar S, Sarathi M, Sarath Babu V, Ramesh B, Sahul Hameed AS. Development and characterization of cell lines derived from rohu, Labeo rohita (Hamilton), and catla, Catla catla (Hamilton). J Fish Dis. 2009a;32:211–8.
3. Ahne W. Persistent infection in CHSE-214 cells with IPN virus isolated from pike (Esox lucius). Bull Off Int Epizoot. 1977;87:415–6.
4. Akimoto K, Takaoka T, Sorimachi K. Development of a simple culture method for the tissues contaminated with microorganisms and application to establishment of a fish cell line. Zool Sci. 2000;17:61–3.
5. Allen DD, Caviedes R, Cárdenas AM, Shimahara T, Segura-Aguilar J, Caviedes PA. Cell lines as in vitro models for drug screening and toxicity studies. Drug Dev Ind Pharm. 2005;31(8):757–68.
6. Anderson DP, Zeeman MG. Immunotoxicology in fish. In: Ran GM, editor. Fundamentals of aquatic toxicology. New York, USA: Taylor and Francis; 1995. p. 371–404.
7. Ariel E, Skall HF, Olesen NJ. Susceptibility testing of fish cell lines for virus isolation. Aquaculture. 2009;298(1–2):125–30.
8. Aarattuthodi S, Dharan V. Applications of fish cell cultures. In Biotechnological advances in aquaculture health management. Singapore: Springer Nature Singapore. 2022:123–163.
9. Ashton I, Clements K, Barrow SE, Secombes CJ, Rowley AF. Effects of dietary fatty acids on eicosanoid-generating capacity, fatty acid composition and chemotactic activity of rainbow trout (Oncorhynchus mykiss) leucocytes. Biochim Biophys Acta. 1994;1214:253–62. https://doi.org/10.1016/0005-2760(94)90071-X.
10. Aviv A, Harley CB. How long should telomeres be? Curr Hypertens Rep. 2001;3:145–51.
11. Babich H, Borenfreund E. Cytotoxicity and genotoxicity assays with cultured fish cells: a review. Toxic in Vitro. 1991;5:91–100.
12. Barker KS, Quiniou SMA, Wilson MR, Bengten E, Stuge TB, Warr GW, Clem LW, Miller NW. Telomerase expression and telomere length in

immortal leukocyte lines from channel catfish. Dev Comp Immunol. 2000;24:583–95.
13. Barman HK, Rasal KD, Chakrapani V, et al. Gene editing tools: state of- the-art and the road ahead for the model and non-model fishes. Transgenic Res. 2017;26(5):577–89.
14. Barman HK, Rasal KD, Mondal S. Status and prospects of gene editing and transgenic in fishes. Indian J Genet Plant Breed. 2019;79(Sup-01):292–9.
15. Behrens A, Schirmer K, Bols NC, Segner H. Polycyclic aromatic hydrocarbons as inducers of cytochrome P4501A enzyme activity in the rainbow trout liver cell line, RTL-W1 and in primary cultures of rainbow trout hepatocytes. Environ Toxicol Chem. 2001;20:632–43.
16. Bejar J, Borrego JJ, Alvarez MC. A continuous cell line from the cultured marine fish gilt-head sea bream (Sparus aurata). Aquaculture. 1997;150:143–53.
17. Bell JG, Sargent JR. The incorporation and metabolism of polyunsaturated fatty acids in phospholipids of cultured cells from chum salmon (Oncorhynchus keta). Fish Physiol Biochem. 1992;10:99–109. https://doi.org/10.1007/BF00004521.
18. Bodnar AG, Ouellette M, Frolkis M, Holt SE, Shiu CP. Extension of lifespan by introduction of telomerase into normal human cells. Science. 1998;279:349–52.
19. Bols NC, Boliska SA, Dixon DG, Hodson PV, Kaiser KLE. The use of fish cell cultures as an indication of contaminant toxicity to fish. Aquat Toxicol. 1985;6:147–55.
20. Bols NC, Barlian AM, Chirino-Trejo M, Caldwell SJ, Goegan P, Lee LEJ. Development of a cell line from primary cultures of rainbow trout, Oncorhynchus mykiss (Walbaum), gills. J Fish Dis. 1994;17:601–11.
21. Bols NC, Lee LEJ. Technology and uses of cell cultures from the tissues and organs of bony fish. Cytotechnology. 1991;6:163–87.
22. Bols NC, Brubacher JL, Ganassin RC, Lee LEJ. Ecotoxicology and innate immunity in fish. Dev Comp Immunol. 2001;25:853–73.
23. Booncherd K, Sreebun S, Pasomboon P, Boonanuntanasarn S. Effects of CRISPR/Cas9-mediated dnd1 knockout impairs gonadal development in striped catfish. Animal. 2024;18(1):101039.
24. Bopp SK, Abicht HK, Knauer K. Copper-induced oxidative stress in rainbow trout gill cells. Aquat Toxicol. 2008;86(2):197–204. https://doi.org/10.1016/j.aquatox.2007.10.014.
25. Brown DW. Threat to humans from virus infections of non-human primates. Rev Med Virol. 1997;7(4):239–46.
26. Bryson SP, Joyce EM, Martell JD, Lee LEJ, Holt SE, Kales SE, Fujiki K, Dixon B, Bols NC. A cell line (HEW) from embryos of haddock (Melanogrammus aeglefinius) and its capacity to tolerate environmental extremes. Mar Biotechnol. 2006;8:641–53.
27. Buchmann K, Nielsen CV, Bresciani J. In vitro interactions between epithelial cells and Gyrodactylus derjavini. J Helminthol. 2000;74(3):203–8.
28. Butler R, Nowak BF. A dual enzyme method for the establishment of long and medium-term primary cultures of epithelial and fibroblastic cells from Atlantic salmon gills. J Fish Biol. 2004;65:1108–25.
29. Caminada D, Zaja R, Smital T, Fent K. Human pharmaceuticals modulate P-gp1 (ABCB1) transport activity in the fish cell line PLHC-1. Aquat Toxicol. 2008;90(3):214–22.
30. Chang N, Sun C, Gao L, Zhu D, Xu X, Zhu X, Xiong JW, Xi JJ. Genome editing with RNA-guided Cas9 nuclease in zebrafish embryos. Cell Res. 2013;23:465–72.
31. Chen MJ, Chiou PP, Liao YH, Lin CM, Chen TT. Development and characterization of five rainbow trout pituitary single-cell clone lines capable of producing pituitary hormones. J Endocrinol. 2010;205:69–78.
32. Chen S, Hong Y, Schartl M. Development of a positive-negative selection procedure for gene targeting in fish cells. Aquaculture. 2002;214:67–79.
33. Chen SL, Sha ZX, Ye HQ. Establishment of a pluripotent embryonic cell line from sea perch blastula embryo. Aquaculture. 2003;218:141–51.
34. Chi SC, HuWW LoBJ. Establishment and characterization of a continuous cell line (GF-1) derived from grouper, Epinephelus coioides: a cell line susceptible to grouper nervous necrosis virus (GNNV). J Fish Dis. 1999;22:173–82.
35. Collet B, Collins C, Lester K. Engineered cell lines for fish health research. Dev Comp Immunol. 2018;80:34–40.
36. Dannevig BH, Falk K, Namork E. Isolation of the causal virus of infectious salmon anaemia (ISA) in a longterm cell line from Atlantic salmon head kidney. J Gen Virol. 1995;76:1353–9.
37. Dayeh VR, Lynn DH, Bols NC. Cytotoxicity of metals common in mining effluent to rainbow trout cell lines and to the] ciliated protozoan. Tetrahymena Thermophila Toxicol In Vitro. 2005;19:399–410.
38. Dayeh VR, Schirmer K, Bols NC. Applying whole-water samples directly to fish cell cultures in order to evaluate the toxicity of industrial effluents. Water Res. 2002;36:3727–38.
39. Dehler CE, Boudinot P, Martin SA, Collet B. Development of an efficient genome editing method by CRISPR/Cas9 in a fish cell line. Mar Biotechnol. 2016;18:449–52.
40. DESPORTES-LIVAGE ISABELLE, Chilmonczyk S, Hedrick R, Ombrouck C, Monge D, Maiga I, GENTILINI M. Comparative development of two microsporidian species: Enterocytozoon bieneusi and Enterocytozoon salmonis, reported in AIDS patients and salmonid fish, respectively. J Eukaryot Microbiol. 1996;43(1):49–60.
41. Devold M, Krossoy B, Asphaug V, Nylund A. Use of RT-PCR for diagnosis of infectious salmon anaemia virus (ISAV) in carrier sea trout Salmo trutta after experimental infection. Dis Aquat Organ. 2000;40:9–18.
42. Dhivyakumari S, Chaudhari A, Brahmane MP, Das DK, Sathiyanarayanan A, Yashwanth BS, Pinto N, Goswami M. Development and characterization of a new muscle cell culture system from Clarias magur (Hamilton, 1822). Fish Physiol Biochem. 2023;49(6):1295–302.
43. Dumont J, Euwart D, Mei B, Estes S, Kshirsagar R. Human cell lines for biopharmaceutical manufacturing: history, status, and future perspectives. Crit Rev Biotechnol. 2016;36(6):1110–22.
44. Dunier M, Siwicki AK. Effects of pesticides and other organic pollutants in the aquatic environment on immunity in fish: a review. Fish Shellfish Immunol. 1993;3:423–38.
45. Ebner HL, Blatzer M, Nawaz M, Krumschnabel G. Activation and nuclear translocation of ERK in response to ligand-dependent and - independent stimuli in liver and gill cells from rainbow trout. J Exp Biol. 2007;210:1036–45.
46. Freshney RI. Culture of animal cells: a manual of basic technique. New Jersey: Wily Interscience; 2005.
47. Fryer JL, Lannan CN. Three decades of fish cell culture: a current listing of cell lines derived from fish. J Tissue Culture Methods. 1994;16:87–94.
48. Furo IDO, Nogueira LS, de Sousa RPC, Oliveira GCS, da Silva DMDS, Malaquias AC, de Oliveira EHC. New parameters for the in vitro development of cell lines from fish species. bioRxiv. 2023:2023–05.
49. Fu X, Li N, Lai Y, Luo X, Wang Y, Shi C, et al. A novel fish cell line derived from the brain of Chinese perch siniperca chuatsi: development and characterization. J Fish Biol. 2015;86(1):32–45.
50. Ganassin RC, Bols NC. Development of a monocyte/ macrophage-like cell line, RTS11, from rainbow trout spleen. Fish Shellfish Immunol. 1998;8:457–76.
51. Genzel Y. Designing cell lines for viral vaccine production: where do we stand? Biotechnol J. 2015;10(5):728–40.
52. Geyer N, Kaminsky S, Confino S, Livne ZBM, Gothilf Y, Foulkes NS, Vallone D. Establishment of cell lines from individual zebrafish embryos. Lab Anim. 2023;57(5):518–28.
53. Goswami M, Pinto N, Yashwanth BS, Sathiyanarayanan A, Ovissipour R. Development of a cell line from skeletal trunk muscle of the fish *Labeo rohita*. Cytotechnology. 2023;75(4):349–61.
54. Goswami M, Yashwanth BS, Trudeau V, et al. Role and relevance of fish cell lines in advanced in vitro research. Mol Biol Rep. 2022;49:2393–411. https://doi.org/10.1007/s11033-021-06997-4.
55. Grist E, Woodhead AD, Carlson C. Established cell lines from nonmammalian vertebrates: models for DNA repair studies. In Vitro Cell Dev Biol. 1986;22:677–80.
56. Hao K, Yuan S, Yu F, Chen XH, Bian WJ, Feng YH, Zhao Z. Acyclovir inhibits channel catfish virus replication and protects channel catfish ovary cells from apoptosis. Virus Res. 2021;292:198249.
57. Harikrishnan R, Balasundaram C, Heo MS. Fish health aspects in grouper aquaculture. Aquaculture. 2011;320(1–2):1–21.
58. Hayflick L, Moorehead PS. The serial cultivation of human diploid cell strains. Exp Cell Res. 1961;25:585–621.
59. He Z, Jiang J, Kokkinaki M, Dym M. Nodal signaling via an autocrine pathway promotes proliferation of mouse spermatogonial stem/

progenitor cells through Smad2/3 and Oct-4 activation. Stem Cells. 2009;27:2580–90.
60. He Z, Jiang J, Kokkinaki M, Golestaneh N, Hofmann MC, Dym M. Gdnf upregulates c-Fos transcription via the Ras/Erk1/2 pathway to promote mouse spermatogonial stem cell proliferation. Stem Cells. 2008;26:266–78.
61. Hedrick RP, Fryer JL. Persistent infection of three salmonid cell lines with infectious pancreatic necrosis virus. IPNVL Fish Pathol. 1981;15:163–72.
62. Hendricks JD, Meyers TR, Casteel JL, Nixon JE, Loveland PM, Bailey GS. Rainbow trout embryos: advantages and limitations for carcinogenesis research. Natl Cancer Inst Mongr. 1984;65:129–37.
63. Hightower LH, Renfro JL. Recent applications of fish cell culture to biomedical research. J Exp Zool. 1988;248:290–302.
64. Holen E, Kausland A, Skjærven K. Embryonic stem cells isolated from Atlantic cod (Gadus morhua) and the developmental expression of a stage-specific transcription factor ac-Pou2. Fish Physiol Biochem. 2010;36:1029–39.
65. Hong N, Li Z, Hong Y. Fish stem cell cultures. Int J Biol Sci. 2011;7(4):392.
66. Hwang WY, Fu Y, Reyon D, Maeder ML, Tsai SQ, Sander JD, Peterson RT, Yeh JJ, Joung JK. Efficient genome editing in zebrafish using a CRISPR-Cas system. Nat Biotechnol. 2013;31(3):227–9.
67. Iqbal G, Ahmad Dar S, Quyoom N, Malik MA, Gul S, Ahmad Mir S, et al. CRISPR/Cas9-Gene editing technology. World J Aquac Res Dev. 2023;3(1):1017.
68. Iqbal G, Quyoom N, Singh LS, Ganpatbhai AVK, Bhat NM, Gul S, Malik MA, Mohanty A, Mir SA, Dar SA. Genome editing technology in fishes. Curr J Appl Sci Technol. 2023;42(23):20–6.
69. Jabbour RE, Snyder AP. Mass spectrometry-based proteomics techniques for biological identification. In biological identification. Woodhead Publishing; 2014:370–430.
70. Jassim Z. Cell Line Culture in Pharmaceutical Development and Application: A Review. Iraqi Journal of Pharmacy. 2023;20(1):17–22.
71. Jeong YJ, Kim KI. A new cell line derived from the caudal fin of the dwarf gourami (Trichogaster lalius) and its susceptibility to fish viruses. Biology. 2023;12(6):829.
72. Jing H, Lin X, Xu L, Gao L, Zhang M, Wang N, Wu S. Establishment and characterization of a heart-derived cell line from goldfish (Carassius auratus). Fish Physiol Biochem. 2017;43(4):977–86.
73. Karunasagr I, Miller SD, Frerichs GN. A new cell line from Puntius schwanenfeldi sensitive to snakehead fish cell line C-type retrovirus. Asian Fish Sci. 1998;8:151–7.
74. Katakura Y, Alam S, Shirahata S. Immortalization by gene transfection. Method Cell Biol. 1998;57:69–91.
75. Kent ML, Speare DJ. Review of the sequential development of Loma salmonae (Microsporidia) based on experimental infections of rainbow trout (Oncorhynchus mykiss) and Chinook salmon (O. tshawytscha). Folia Parasitol (Praha). 2005;52(1–2):63–68.
76. Kimura T, Yoshimizu M, Tanaka M, et al. Fish viruses: tumor induction in Oncorhynchus keta by the herpesvirus. In: Dawe CJ, et al., editors. Phyletic approaches to cancer. Tokyo: Jpn Sci Soe Press. 1981:59–68.
77. Kimelman D, Kirschner M. Synergistic induction of mesoderm by FGF and TGF-B and the identification of an mRNA coding for FGF in the early Xenopus embryo. Cell. 1987;51:869–77.
78. Kishimoto K, Washio Y, Yoshiura Y, Toyoda A, Ueno T, Fukuyama H, Kinoshita M. Production of a breed of red sea bream Pagrus major with an increase of skeletal muscle mass and reduced body length by genome editing with CRISPR/Cas9. Aquaculture. 2018;495:415–27.
79. Kiyono T, Foster SA, Koop JI, McDougall JK, Galloway DA. Both Rb/p16INK4a inactivation and telomerase activity are required to immortalize human epithelial cells. Nature. 1998;396:84–8.
80. Kocan RM, Sabo KM, Landolt ML. Cytotoxicity-genotoxicity: the application of cell culture techniques to the measurement of marine sediment pollution. Aquat Toxicol. 1985;6:165–78.
81. Krumschnabel G, Maehr T, Nawaz M, Schwarzbaum PJ, Manzl C. Staurosporine-induced cell death in salmonid cells: the role of apoptotic volume decrease, ion fluxes and MAP kinase signaling. Apoptosis. 2007;12(10):1755–68.
82. Kumar GS, Singh IBS, Philip R. Development of a cell culture system from the ovarian tissue of African catfish (Clarias gariepinus). Aquaculture. 2001;194:51–62.
83. Lakra WS, Bhonde RR. Development of primary cell culture from the caudal fin of an Indian major carp, Labeo rohita (Ham). Asian Fish Sci. 1996;9:149–52.
84. Lakra WS, Sivakumar N, Goswami M, Bhonde RR. Development of two cell culture systems from Asian seabass Lates calcarifer (Bloch). Aquacult Res. 2006b;37:18–24.
85. Lakra WS, Swaminathan TR, Joy KP. Development, characterization, conservation and storage of fish cell lines: a review. Fish Physiol Biochem. 2011;37:1–20. https://doi.org/10.1007/s10695-010-9411-x.
86. Lannan CN, Winton JR, Fryer JL. Fish cell lines: establishment and characterization of nine cell lines from salmonids. In vitro. 1984;20(9):671–6.
87. Lee LE, Van Es SJ, Walsh SK, Rainnie DJ, Donay N, Summerfield R, Cawthorn RJ. High yield and rapid growth of Neoparamoeba pemaquidensis in co-culture with a rainbow trout gill-derived cell line RTgill-W1. J Fish Dis. 2006;29:467–80.
88. Li C, Fu X, Lin Q, Liu L, Liang H, Huang Z, Li N. Autophagy promoted infectious kidney and spleen necrosis virus replication and decreased infectious virus yields in CPB cell line. Fish Shellfish Immunol. 2017;60:25–32.
89. Li N, Guo L, Guo H. Establishment, characterization, and transfection potential of a new continuous fish cell line (CAM) derived from the muscle tissue of grass goldfish (*Carassius auratus*). In Vitro Cell Dev Biol Anim. 2021;57:912–31.
90. Li H, Zhang S. In vitro cytotoxicity of the organophosphorus insecticide methylparathion to FG-9307, the gill cell line of flounder (Paralichthys olivaceus). Cell Biol Toxicol. 2002;18(4):235–41. https://doi.org/10.1023/A:1016050911012.
91. Li H, Zhang S. In vitro cytotoxicity of the organophosphorus pesticide parathion to FG-9307 cells. Toxicol In Vitro. 2001;15(6):643–7.
92. Lin CY, Chiang CY, Tsai HJ. Zebrafish and Medaka: new model organisms for modern biomedical research. J Biomed Sci. 2016;23:1–11.
93. Liu Q, Yuan Y, Zhu F, Hong Y, Ge R. Efficient genome editing using CRISPR/Cas9 ribonucleoprotein approach in cultured Medaka fish cells. Biol Open. 2018;7(8):bio035170.
94. Loveland PM, Wilcox JS, Pawlowski NE, Bailey GS. Metabolism and DNA binding of aflatoxicol and aflatoxin B1 in vivo and in isolated hepatocytes from rainbow trout (Salmo gairdneri). Carcinogenesis. 1987;8:1065–70.
95. Mansour SL, Thomas KR, Capecchi MR. Disruption of the proto-oncogene int-2 in mouse embryo-derived stem cells: a general strategy for targeting mutations to non-selectable genes. Nature. 1988;336:348–52.
96. Marion M, Denizeau F. Rainbow trout and human cells in culture for the evaluation of the toxicity of aquatic pollutants: a study with lead. Aquat Toxicol. 1983;3:47–60.
97. Martin BJ, Ellender RD, Hillebert SA, Guess MM. Primary cell cultures from the teleost, Cyprinodon uariegatus. Culture establishment and application in carcinogen exposure studies. Natl Cancer Inst Mongr. 1984;65:175–8.
98. Masters JRW, Thomson JA, Daly-Burns B, Reid YA, Dirks WG, Packer P, Toji LH, Ohno T, Tanabe H, Arlett CF, Kelland LR, Harrison M, Virmani A, Ward TH, Ayres KL, Debenham PG. Short tandem repeat profiling provides an international reference standard for human cell lines. Proc Natl Acad Sci U S A. 2001;98:8012–7.
99. Matsuo Y, Nishizaki C, Drexler HG. Efficient DNA fingerprinting method for the identification of cross-culture contamination of cell lines. Hum Cell. 1999;12:149–54.
100. Morin G, Pinel K, Dias K, Seiliez I, Beaumatin F. RTH-149 cell line, a useful tool to decipher molecular mechanisms related to fish nutrition. Cells. 2020;9(8):1754.
101. Nims L, Fryer JL, Pilcher KS. Studies of replication of four selected viruses in two cell lines derived from salmonid fish. Proc Soc Exp Biol Med. 1970;135:6–12.
102. Noga EJ. Propagation in cell culture of the dinoflagellate Amyloodinium, an ectoparasite of marine fishes. Science. 1987;236(4806):1302–4. https://doi.org/10.1126/science.236.4806.1302.
103. Oh SY, Kim WS, Oh MJ, Nishizawa T. Multiplication rate of red seabream iridovirus (RSIV) in rock bream Oplegnathus fasciatus at different fish rearing temperatures. Fish Pathol. 2016;51(4):194–8.
104. Okocha RC, Olatoye IO, Adedeji OB. Food safety impacts of antimicrobial use and their residues in aquaculture. Public Health Rev. 2018;39:1–22.

105. Ottinger CA, Kaattari SL. Long-term immune dysfunction in rainbow trout (Oncorhynchus mykiss) exposed as embryos to aflatoxin B1. Fish Shellfish Immunol. 2000;10:101–6.
106. Pandey G. Overview of fish cell lines and their uses. Int J Pharm Res Sci. 2013;2:580–90.
107. Parameswaran V, Ahmed VPI, Shukla R, Bhonde RR, Hameed ASS. Development and characterization of two new cell lines from milkfish (Chanos chanos) and grouper (Epinephelus coioides) for virus isolation. Mar Biotechnol. 2007;9:281–91.
108. Pauwels K, Herman P, Van sVaerenbergh B, Dai Do Thi C, Berghmans L, Waeterloos G, Sneyers M. Animal cell cultures: risk assessment and biosafety recommendations. Appl Biosafety. 2007;12(1):26–38.
109. Perry GML, McDonald GJ, Ferguson MM, Ganassin RC, Bols NC. Characterization of rainbow trout cell lines using microsatellite DNA profiling. Cytotechnology. 2001;37:143–51.
110. Pilcher KS, Fryer JL. The viral diseases of fish: a review through 1978. CRC Crit Rev Microbiol. 1980;7:287–364.
111. Pombinho AR, Laize V, Molha DM, Marques SMP, Cancela LM. Development of two bone-derived cell lines from the marine teleost Sparus aurata; evidence for extra cellular matrix mineralization and cell-type-specific expression of matrix Gla protein and osteocalcin. Cell Tissue Res. 2004;315:393–406.
112. Powell RL, Dodson MV, Cloud JG. Cultivation and differentiation of satellite cells from skeletal muscle of the rainbow trout *Salmo gairdneri*. J Exp Zool. 1989;250(3):333–8.
113. Ristow SS, De Avila J. Susceptibility of four salmonids cell lines to infectious hematopoietic necrosis virus. J Aquat Anim Health. 1994;6:260–5.
114. Rosa F, Roberts AB, Danielpour D, Dart LL, Sporn MB, David IB. Mesoderm induction in amphibians: the role of TGF-p2-like factors. Science. 1988;239:783–5.
115. Rubio N, Datar I, Stachura D, Kaplan D, Krueger K. Cell-based fish: a novel approach to seafood production and an opportunity for cellular agriculture. Front Sustain Food Syst. 2019;3:43.
116. Sathe PS, Basu A, Mourya DT, Marathe BA, Gogate SS, Banerjee K. A cell line from the gill tissues of Indian cyprinoid Labeo rohita. In Vitro Cell Dev Biol Anim. 1997;33:425–7.
117. Sathe PS, Maurya DT, Basu A, Gogate SS, Banerjee K. Establishment and characterization of a new fish cell line, MG-3, from the gills of mrigal Cirrhinus mrigala. Ind J Exp Biol. 1995;33:589–94.
118. Sato A, Okamoto N. Induction of virus-specific cell-mediated cytotoxic responses of isogeneic ginbuna crucian carp, after oral immunization with inactivated virus. Fish Shellfish Immunol. 2010;29(3):414–21.
119. Schirmer K, Chan AGJ, Greenberg BM, Dixon DG, Bols NC. Ability of 16 priority PAHs to be photocytotoxic to a cell line from the rainbow trout gill. Toxicology. 1998;127:143–55.
120. Schirmer K, Dixon DG, Greenberg BM, Bols NC. Ability of 16 priority PAHs to be directly cytotoxic to a cell line from the rainbow trout gill. Toxicology. 1998;127:129–41.
121. Schirmer K, Herbrick JAS, Greenberg BM, Dixon DG, Bols NC. Use of fish gill cells in culture to evaluate the cytotoxicity and photocytotoxicity of intact and photomodified creosote. Environ Toxicol Chem. 1999;18:1277–88. https://doi.org/10.1897/15515028(1999)018%3c1277:UOFGCI%3e2.3.CO;2.
122. Schirmer K, Tom DJ, Bols NC, Sherry JP. Ability of fractionated petroleum refinery effluent to elicit cyto- and photocytotoxic responses and to induce 7-ethoxyresorufin-Odeethylase activity in fish cell lines. Sci Total Environ. 2001;271(1–3):61–78.
123. Scholz S, Segner H. Induction of CYP1A in primary cultures of rainbow trout (Oncorhynchus mykiss) liver cells: concentration-response relationships of four model substances. Ecotoxicol Environ Saf. 1999;43:252–60.
124. Servili A, Bufalino MR, Nishikawa R, de Melo IS, Mun˜oz- Cueto JA, Lee LEJ,. Establishment of long term cultures of neural stem cells from adult sea bass, Dicentrarchus labrax. Comp Biochem Physiol Part A. 2009;152:245–54.
125. Sha ZA, Ren G, Wang X, Wang N, Chen S. Development and characterization of a cell line from the embryos of half smooth tongue sole (Cynoglossus semilaevis). Acta Oceanol Sin. 2010;29(2):81–7.
126. Shao J, Eckert ML, Lee LEJ, Gallagher EP. Comparative oxygen radical formation and toxicity of BDE 47 in rainbow trout cell lines. Mar Environ Res. 2008;66:7–8. https://doi.org/10.1016/j.marenvres.2008.02.007.
127. Sinnhuber RO, Hendricks JD, Wales JH, Putnam GB. Neoplasms in rainbow trout, a sensitive animal model for environmental carcinogenesis. Ann N Y Acad Sci. 1977;298(1):389–408.
128. Sobhana KS. Development of marine fish cell lines and stem cell lines: applications in mariculture and marine biodiversity. 2015.
129. Speare DJ, Markham RJ, Guselle NJ. Development of an effective whole-spore vaccine to protect against microsporidial gill disease in rainbow trout (Oncorhynchus mykiss) by using a low-virulence strain of Loma salmonae. Clin Vaccine Immunol. 2007;14(12):1652–4.
130. Swaminathan TR, Kumar R, Jency PME, Charan R, Syamkrishnan MU, Basheer VS, et al. A new fish cell line derived from the caudal fin of freshwater angelfish Pterophyllum scalare: development and characterization. J Fish Biol. 2016;89(3):1769–81.
131. Swaminathan TR, Lakra WS, Gopalakrishnan A, Basheer VS, Khushwaha B, Sajeela KA. Development and characterization of a new epithelial cell line PSF from caudal fin of green chromide, Etroplus suratensis (Bloch, 1790). In Vitro Cell Dev Biol Anim. 2010;46:647–56.
132. Takakura M, Kyo S, Kanaya T, Hirano H, Takeda J, Yutsudo M, Inoue M. Cloning of human telomerase catalytic subunit (hTERT) gene promoter and identification of proximal core promoter sequences essential for transcriptional activation in immortalized and cancer cells. Cancer Res. 1999;59:551–7.
133. Tao B, Tan J, Chen L, Xu Y, Liao X, Li Y, Chen J, Song Y, Hu W. CRISPR/Cas9 system-based myostatin-targeted disruption promotes somatic growth and adipogenesis in loach. Misgurnus anguillicaudatus Aquaculture. 2021;544: 737097.
134. Tong SL, Lee H, Miao HZ. The establishment and partial characterization of a continuous fish cell line FG- 9307 from the gill of flounder Paralichthys olivaceus. Aquaculture. 1997;156:327–33.
135. Van Beneden RJ, Watson DK, Chen TT, Lautenberger JA, Papas TS. Cellular myc (c-myc) in fish (rainbow trout): Its relationship to other vertebrate myc genes and to the transforming genes of the MC29 family of viruses. Roc Natl Acad Sci USA. 1986;83:3698–702.
136. Villena AJ. Applications and needs of fish and shellfish cell culture for disease control in aquaculture. Rev Fish Biol Fisheries. 2003;13:111–40.
137. Wagg SK, Lee LEJ. A proteomics approach to identifying fish cell lines. Proteomics. 2005;5:4236–44.
138. Wang G, LaPatra S, Zeng L, Zhao Z, Lu Y. Establishment, growth, cryopreservation and species of origin identification of three cell lines from white sturgeon, Acipenser transmontanus. Methods Cell Sci. 2003;25:211–20.
139. Wang N, Wang XL, Sha ZX, Tian YS, Chen SL. Development and characterization of a new marine fish cell line from turbot (Scophthalmus maximus). Fish Physiol Biochem. 2010;36:1227–34.
140. Wang H, Wen L, Yuan Q, Sun M, Niu M, He Z. Establishment and applications of male germ cell and Sertoli cell lines. Reproduction. 2016;152(2):R31–40.
141. Wang J, Yu X, Zhao S, Zhang N, Lin Z, Wang Z, Ma J, Yan Y, Sun J, Cheng Y. Construction of a peacock immortalized fibroblast cell line for avian virus production. Poult Sci. 2022;101(12):102147.
142. Wang Q, Fang J, Pan Q, Wang Y, Xue T, Li L, Chen T. Efficient and stable delivery of multiple genes to fish cells by a modified recombinant baculovirus system. Int J Mol Sci. 2018;19(12):3767.
143. Weeks DL, Melton DA. A maternal mRNA localized to the vegetal hemisphere in Xenopus eggs codes for a growth factor related to TGF-B. Cell. 1987;51:2361–867.
144. Wenger SL, Senft JR, Sargent LM, Bamezai R, Bairwa N, Grant SG. Comparison of established cell lines at different passages by karyotype and comparative genomic hybridization. Biosci Rep. 2004;24:631–9.
145. Wergeland HI, Jakobsen RA. A salmonid cell line (TO) for production of infectious salmon anaemia virus (ISAV). Dis Aquat Organ. 2001;44:183–90.
146. Winton JR, Lannan CN, Fryer JL, Kimura T. Isolation of a new reovirus from chum salmon in Japan. Fish Pathol. 1981;15:155–62.
147. Wolf K, Darlington RW. Channel catfish virus: a new herpesvirus of ictalurid fish. J Virol. 1971;8(4):525–33.
148. Wolf K, Darlington RW, Taylor WG, Quimby MC, Nagabayashi T. Herpesvirus salmonis: characterization of a new pathogen of rainbow trout. J Virol. 1978;27:659–66.
149. Wolf K, Mann JA. Poikilotherm vertebrate cell lines and viruses: a current listing for fishes. In Vitro. 1980;16:168–79.

150. Wolf K, Ahne W. Fish cell culture. Adv Cell Cult. 1982;2:305–28 Elsevier.
151. Wu Y, Wu T, Yang L, Su Y, Zhao C, Li L, Cai J, Dai X, Wang D, Zhou L. Generation of fast growth Nile tilapia (Oreochromis niloticus) by myostatin gene mutation. Aquaculture. 2023;562:738762.
152. Yao C, Liu Y, Sun M, Niu M, Yuan Q, Hai Y, Guo Y, Chen Z, Hou J, He Z. MicroRNAs and DNA methylation as epigenetic regulators of mitosis, meiosis and spermiogenesis. Reproduction. 2015;150:R25–34.
153. Yashwanth BS, Goswami M, Kooloth Valappil R, Thakuria D, Chaudhari A. Characterization of a new cell line from ornamental fish Amphiprion ocellaris (Cuvier, 1830) and its susceptibility to nervous necrosis virus. Sci Rep. 2020;10(1):20051.
154. Yashwanth BS, Pinto N, Sathiyanarayanan A, Chaudhari A, Rasal KD, Goswami M. Functional characterization of Labeo rohita muscle cell line for in vitro research. Mol Biol Rep. 2023;50(7):5635–46.
155. Yang Y, Ren Y, Zhang Y, Wang G, He Z, Liu Y, Cao W, Wang Y, Chen S, Fu Y, Hou J. A new cell line derived from the spleen of the Japanese Flounder (*Paralichthys olivaceus*) and its application in viral study. Biology. 2022;11(12):1697–1697.
156. Zeeman MG, Brindley WA. Effects of toxic agents upon fish immune system: a review. In: Sharma RP, editor. Immunological Considerations in Toxicology, vol. 2. CRC Press. Florida: Boca Raton; 1981. p. 1–60.
157. Zelikoff JT. Metal pollution induced immunomodulation in fish. Ann Rev Fish Dis. 1993;3:305–25.
158. Zhang CL, Huang T, Wu BL, He WX, Liu D. Stem cells in cancer therapy: opportunities and challenges. Oncotarget. 2017;8(43):75756.
159. Zhao Y, Glesne D, Huberman E. A human peripheral blood monocyte derived subset acts as pluripotent stem cells. Proc Natl Acad Sci. 2003;100:2426–31.
160. Zhong Z, Niu P, Wang M, Huang G, Xu S, Sun Y, Wang H. Targeted disruption of sp7 and myostatin with CRISPR-Cas9 results in severe bone defects and more muscular cells in common carp. Scientific Reports. 2016;6(1):22953.

Publisher's Note