The Zebrafish model in dermatology: an update for clinicians

Irene Russo · Emma Sartor · Laura Fagotto · Anna Colombo · Natascia Tiso · Mauro Alaibac

Received: 23 May 2022 / Accepted: 8 June 2022
Published online: 17 June 2022
© The Author(s) 2022  OPEN

Abstract
Recently, the zebrafish has been established as one of the most important model organisms for medical research. Several studies have proved that there is a high level of similarity between human and zebrafish genomes, which encourages the use of zebrafish as a model for understanding human genetic disorders, including cancer. Interestingly, zebrafish skin shows several similarities to human skin, suggesting that this model organism is particularly suitable for the study of neoplastic and inflammatory skin disorders. This paper appraises the specific characteristics of zebrafish skin and describes the major applications of the zebrafish model in dermatological research.

1 Introduction
Since their use, animal model systems have offered a technical means to perform studies that could not be otherwise undertaken in human subjects. They represent a fundamental part of research and offer precious keys to understanding human physiology and pathophysiology. Moreover, not only do they allow for more advanced pharmacokinetic and pharmacodynamic studies, but also for the discovery of new treatments for human diseases.

Because of their well-known physiological and genetic similarities to humans, murine and other mammalian models have been routinely used for medical research. However, other animal models are showing distinct advantages over these conventional models. Therefore, interest in this field has increased over recent years.

Among the animal models studied thus far, fish seem to be the most interesting non-mammalian vertebrate model, because of their low maintenance costs and ex utero development of progeny that allows for in vivo imaging. For instance, Platyfish (Xiphophorus) and Medaka (Oryzias latipes) have already been successfully used as models to study melanoma [1–3].

2 The zebrafish organism
The zebrafish (Danio rerio) was first introduced as a model for genetic studies by Streisinger and colleagues in the early 1980s [4]. Zebrafish are small vertebrate tropical fish characterized by low-cost maintenance and exploited as a model for both reverse and forward genetic studies.
At first, zebrafish were used as a model in forward genetic studies (the identification of a specific genotype through observation of a certain phenotype). This was the case in N-ethyl-N-nitrosurea (ENU) induced mutagenesis used to produce point mutations, followed by extensive phenotypic screening. However, forward genetic screens using this method were time consuming and laborious [5, 6].

Fortunately, increasingly advanced techniques in recent years have allowed to overcome the challenges of creating several disease models using zebrafish and to expand zebrafish studies through the reverse genetics (the observation of the phenotype produced by a known genotype). Moreover, studies have proved that there is a high level of similarity between human and zebrafish genomes, estimating that 70% of human genes have at least one zebrafish orthologue. These data are astonishing, especially if we consider that a mouse shares about 80% of its genome with humans [7, 8]. Moreover, over 80% of known human disease genes, including oncogenes and tumor suppressor genes, have their orthologues in zebrafish and several pathways are also conserved, even those implicated in carcinogenesis [8].

All these characteristics and evidence have boosted the use of zebrafish as a model for understanding human genetic disorders, including cancer, and have pointed out their potential for in vivo screening for new therapies, which is especially important in our era of personalized medicine.

Therefore, although mouse models remain the most used in the medical research field, the zebrafish has several advantages and unique features that murine models do not have, which explain its ancillary and complementary role.

The advantages of using zebrafish as a model are numerous, going beyond their low-cost maintenance and small size. They display a high fecundity, with the ability to fertilize about 200–300 eggs every 5–7 days. Fast ex utero development together with the embryos’ optical clarity allow for the observation of early physiological and/or pathological development by in vivo direct cell imaging [9, 10]. In addition, the casper zebrafish was introduced as a genetic strain intentionally created to maintain transparency throughout adult life, making it even more affordable to study cancer cells’ behavior in a living organism [11].

Cell-based assays for the study of the absorption, distribution, metabolism and toxicity of compounds and drugs give only limited information, whereas pharmacologic molecule screening in zebrafish might help to overcome this problem. Parallel physiological responses have been observed in the use of drugs and small molecules in zebrafish and mammal models [9]. Drug screenings benefit from both the embryos’ transparency, which makes it easy to collect imaging data after treatment, and the high throughput assays, which are made possible by the female's ability to lay many eggs (about 10,000 eggs per annum). This means that imaging, cellular analysis and advanced statistics can be performed simultaneously in an incredibly large number of fish, with laboratory space being the only limiting factor [12, 13]. These premises explain the zebrafish’s potential role as a bridge between cell-based assays and biological validation of a certain compound.

Moreover, zebrafish might represent a clue in the attempt to identify therapeutic targets for the treatment of human diseases, which remains a big challenge in medical science [14]. In phenotype-guided drug studies, the presence of phenotype alterations in the whole organism may suggest the effectiveness of a drug, even when the target is unknown. This approach helps both the development of new drugs and the simultaneous identification of the molecular pathways underlying the disease that are inducing that specific phenotype [15]. Compared to mice, zebrafish studies enable us to analyze a greater number of phenotypes at reduced costs and labor.

Even though this fish model is extremely versatile in medical and especially in pharmacological research, there are a few drawbacks that should also be pointed out. Firstly, the zebrafish is a poikilothermic fish that needs to be bred in an environment with a temperature around 28 °C to survive. This differs from mammals’ homeostatic temperature, thus hindering studies where temperature is a determining factor. However, it can tolerate a wide range of temperature variation, spanning between 6 and 38 °C, for limited periods of time [16]. Secondly, teleost genome duplication involves the presence of genes in more than one copy (paralogs), which might hamper molecular genetic studies. Lastly, another disadvantage of the zebrafish is the scarcity of available antibodies that specifically target zebrafish proteins, and the technical difficulty in raising antibodies against zebrafish targets [17–19]. This is especially relevant for cell surface and secreted proteins, since immunogenic glycans on zebrafish extracellular proteins hamper elicitation of protein-specific antibodies in mammals used for raising such antibodies [20].

### 2.1 Zebrafish skin

Fish skin comprises the epidermis, dermis, and hypodermis, thus resembling mammalian skin. However, unlike mammals’ and terrestrial vertebrates’ epidermis, which is covered by an outer layer of keratinized dead cells, zebrafish skin surface is made of living cells that are covered with mucus and lacks a cornified envelope [21]. Furthermore, zebrafish
has no mammalian appendages, since hair follicles and sebaceous glands cannot be detected. However, zebrafish presents the breeding tubercle, which is an epidermal appendage shared with mammals [22].

Mammalian epidermis is a well-organized stratified tissue that includes basal, spinous, granular, and horny cells from the basal membrane to the skin surface. Teleost epidermis only has three layers [23]. The surface layer is a single cell layer in which cells are rich in keratin filaments and are continuously replaced at their death, without producing a stratum corneum. The intermediate layer is composed of different cell types, including unicellular glands (mucous cells and club cells), sensory cells, ionocytes and undifferentiated cells. The basal layer is a single cell layer which is attached to the basement membrane via hemidesmosomes, which tightly link the epidermis to the dermis [24].

Maturation of zebrafish from embryo to fully developed fish only takes a few days. Layers representing the epidermis and the dermis are already detectable at one day post-fertilization (dpf). In adult zebrafish, scales covering the epidermis form at around the 30th dpf and sonic hedgehog pathway has been identified as having a role in their development [25]. Collagenous stroma formation is dependent on fibroblasts, whereas pigment production derives from melanocytes, belonging to neural crest-derived pigment cell system [26].

Several epidermal marker genes, including keratins 1 and 5, the 230 kDa bullous pemphigoid antigen, plectin, and several cutaneous basement membrane zone (BMZ) genes, including type IV, VII and XVII collagen, are expressed in zebrafish skin in early developmental stages. Most expressed human collagens types, including collagens I, V, and VI, are detectable in zebrafish skin from 6th dpf. In conclusion, zebrafish repertoire of genes involved in cutaneous development reveals strong similarities with human skin [25].

The zebrafish neural crest produces three different kinds of pigment cells: melanophores, xanthophores and iridophores (Fig. 1). Melanophores synthesize melanin and are analogous to melanocytes of vertebrates, xanthophores have a yellow appearance caused by pteridine pigments, and iridophores contain iridescent platelets which reflect light. Melanophores firstly develop among pigmented cells at approximately 24 h post fertilization from melanogenic progenitors deriving from the neural crest [27].

Like in mammalian melanocytes, the tyrosine-protein kinase KIT has a major role in promoting the initial migration of melanocytes in the first two days of the embryos [28]. Later in zebrafish larval development, a new set of melanocytes contribute to formation of stripes characterizing adult zebrafish after metamorphosis [29].

### 2.2 Skin inflammation in zebrafish

Skin is essential in defending fish from environmental stress factors. Since fish are poikilotherms, even small changes in the external parameters may lead to injury and inflammation [30]. A series of epithelial cells, resident non-immune cells, vascular endothelial cells and mucosal epithelial cells help initiate and coordinate the inflammatory response [31–33]. Interestingly, unlike humans, [34], fish do not have major lymphoid accumulations. It is still unclear where
fish lymphoid cells naturally reside. The most probable theory is that fish leukocytes migrate to the skin via mucus secretions in response to damage stimuli [35–37].

Inflammation pathways are regulated by the NF-κB family transcription factor both in mammals and fish [38] and classic pro-inflammatory cytokines such as IL-1β, TNF-α and IL-6 prevail as paralogues in most teleosts [39–41], thus making the main mechanisms of inflammation similar in the two species. Neutrophils play an essential role in initiating the inflammatory response in both mammals and zebrafish and in perpetration of the inflammation, especially via TNF-α and IL-1β [42–48].

Once activated, monocytes differentiate into classically pro-inflammatory macrophages, functioning as antigen presentation cells and producing reactive oxygen species (ROS), TNF-α and IL-1β [42, 49–52].

Besides, neutrophils activation determines exocytosis of their granules [42, 50, 51, 53, 54]. Antigen presenting cells such as dendritic cells, macrophages and endothelial cells are also recruited by neutrophils [51]. While in mammals leukocytes originate in the bone marrow and mature in lymph nodes, zebrafish lacks such structures [55]. Specifically, the bone marrow has its counterpart in the head kidney acting as a major hematopoietic and lymphoid organ. Indeed, the thymus, spleen and mucosa-associated lymphoid tissues (MALT) are shared between fish and mammals [56]. Migration and proliferation of the immune cells in zebrafish skin has been recently studied using fluorescent light which induced the early expression of skin genes associated with inflammation [57].

3 Zebrafish as a model system in oncology

One of the biggest obstacles in the field of oncology is to address cancer heterogeneity either in inter-individual differences or in intra-tumoral contexts [58, 59]. Innovations in cancer research have largely benefited from further exploring these processes in live animals, with a strive to identify and target the most frequent driver mutations as a rational approach to treatment [60]. This is especially done in early tumor development at a cellular level.

As an example, patient-derived cancer cell xenotransplantation (PDX) could help to overcome treatment resistance due to added mutations in tumor cells, by means of large-scale drug (small molecules) screening. This process is not as easily achievable in murine models as it may be in non-mammalian models such as fish, since recipient immunosuppression is required for PDX in mice [61–63].

The zebrafish has recently caught attention due to the aforementioned characteristics, alongside its highly-conserved cancer signaling pathways compared to the human species.

Moreover, the zebrafish is a versatile model, as it is possible to operate a mutation in a specific gene, thus creating a stable transgene, or to create a transient over-expression or down-regulation of a specific gene. Forward and reverse genetic screens are also possible in zebrafish [64].

Initially, zebrafish have been used in forward genetic screens to test the effects of mutagens on neoplasm development. Ethylnitrosurea screens leading to mutations in tp53, one of most studied genes among those involved in cancer pathogenesis, were among the first experiments in zebrafish in the field of oncology [65, 66].

Further techniques have been subsequently introduced, which helped cancer studies in zebrafish to progress. The aim was to create loss-of-function phenotypes or to introduce transgenes that are typically mutated in human cancer into fish models. Research in this field brought forward evidence that many mutated tumor suppressor genes, such as Tp53, and oncogenes, such as mMyC and KRas, could generate parallel tumors in zebrafish in the same way as they had been observed in humans. This shed light on the evolutionary conservation of drivers and pathways of tumorigenesis between man and fish [67].

Currently, the most used techniques for gene manipulation in zebrafish are morpholino oligomers (MOs) [68], zinc finger nucleases (ZFNs) [69], transcription activator-like effector nucleases (TALENs) [70] and the CRISPR (clustered regularly interspaced short palindromic repeats) system [71]. However, new promising techniques have recently been introduced to be used in zebrafish, such as TEAZ (transgene electroporation in adult zebrafish) and tumor cell transplantation, especially in the form of PDX (patient-derived cancer cell xenotransplantation).

MOs are small synthetic oligomers that block mRNA translation in vivo; they are easy to use and enable us to obtain models in a short amount of time, despite concerns about their off-target effects and their inexact reproduction of genome-editing mutants, thus requiring a control with the knockout phenotype [68, 72, 73].

ZFNs is a useful technique for multiplex gene targeting to be performed in one round, either creating knock-outs (loss of function) or knock-ins (gain of function) [69].
TALENs enable us to produce heritable gene disruptions in the vertebrate genome; more importantly, they can create mutations in somatic tissues with a high success rate, including bi-allelic mutations [70].

CRISPR/Cas9 is a technique in which Cas9 endonuclease recognizes a specific DNA sequence by means of a guide RNA sequence binding both DNA and Cas9. Zebrafish models based on this technique are widely used today due to the potential possibility to target multiple genes at the same time and to its high efficiency [71, 74, 75].

TEAZ is a new technique that enables the injection of DNA constructs containing tissue-specific promoters and genes of interest into adult tissue. In addition, TEAZ is extremely fast as far as tumor onset is concerned, and the expression of genes of interest can be evaluated in adult fish [76]. TEAZ is very promising compared to conventional zebrafish cancer models created by means of the aforementioned techniques. In fact, the latter involves the injection of nucleic acids into one-cell stage embryos. Therefore, it is sometimes difficult to study cancer pathogenesis and development in animal models, since the onset and the site of the developing tumors are not accurate, and the spreading of metastases could be hard to evaluate. Alongside TEAZ, cancer cell transplantation in zebrafish embryos and adults could partially overcome the problems connected with common techniques [77].

Tumor cell transplantation is an important tool in studying tumor invasiveness. It involves cancer cell transplantation from a donor to a recipient of the same species (allograft) or of a different species (xenograft) [78].

Many studies have demonstrated that zebrafish embryos can engraft human cancer cells and give precious insight into disease pathogenesis. As for human cancer xenotransplantation, zebrafish have some advantages compared to murine models, especially because a high number of transparent embryos lacking a mature immune system can be transplanted with cancer cells and tracked. In other words, visualization of cell-cell interactions in vivo is possible in zebrafish. Moreover, PDX in zebrafish can help us find new targets for targeted anti-cancer treatments. There is evidence that pre-clinical research might shorten the time for drug approval, mostly due to drug re-purposing [10]. The zebrafish has already shown to be a reliable model to assess drug efficacy and sensitivity, since in some experiments patient-derived cells responded well to the same drugs that were used in patients [79].

Thus, the use of zebrafish as a pre-clinical screening model for patient-derived cancer cell xenotransplantation might revolutionize our approach to cancer, especially in a personalized medicine perspective, and explains the growing interest in PDX studies in zebrafish [77].

The variety of cancer types that have been successfully reproduced in zebrafish prove that this animal model has a lot of potential in the analysis of almost every type of cancer observed in humans. Genetic models of cancer in zebrafish include peripheral nerve sheath tumor (PNST) [80–82], rhabdomyosarcoma (RMS) [83, 84], melanoma, [85–90] thyroid cancer [91], pancreatic cancer [92, 93], hepatocellular carcinoma (HCC) [94–96], intestinal tumors [97, 98], testicular tumors [99], T-cell acute lymphoid leukemia (T-ALL) [83, 100–102], Acute Lymphoid leukemia (AML) [103–106], chronic myeloid leukemia (CML) [102, 107], myelodysplastic syndrome (MDS) [108].

Some of these cancer types, along with others, have been studied with PDX in Danio rerio [10]. Interestingly, the zebrafish has proved to be a reliable model for PDX for some cancers that develop in human organs that fish do not have, such as the breast, prostate and lungs [109, 110]. It has proved to be a good model for studying rare cancer pathogenesis as well, such as Ewing sarcoma [111].

### 3.1 Melanoma models in zebrafish

To better understand the mechanisms underlying melanoma, the zebrafish represents an excellent model through the use of xenografts [112] and transgenic models [113, 114].

Melanoma has certainly been one of the most studied cancers and the most analyzed skin cancer in zebrafish, since the first description of BRAF V600E model. It is known that the V600E mutation, a key melanoma driver found in about 43–50% of melanomas [10, 115, 116], is also frequently found in benign naevi and moles which do not progress to cancer. It is also known that the loss of function of the tumor suppressor gene p53 (p53−/−) is required for cancer progression in naevi. However, the long time lapse and rarity of melanoma tumor formation (one to three in a fish’ lifetime) in zebrafish carrying both BRAF V600E and p53−/− mutations, imply that there are other molecular alterations and pathways playing a role in melanoma formation. Based on the observation that crestin, the expression of which is generally limited to neural crest progenitor cells in developing zebrafish embryos, was expressed in zebrafish melanomas [117], studies were performed in which engineered transgenic zebrafish expressing GFP (green fluorescent protein) under the control of crestin-regulatory elements were tracked. GFP-positive cells showed that only individual melanocytes that reactivated crestin could initiate melanomas. This highlighted that melanoma at a one-cell-state is based on reprogramming the
cell to become more neural-crest-like [86]. As confirmation, consistent with crestin expression was the expression of the SOX10 transcription factor, a conserved early neural crest marker that helps melanocyte reprogramming to an embryonic state. Reactivation of neural crest genes such as crestin (in zebrafish melanoma) and SOX10 (in zebrafish as well as in human melanoma cell lines) is probably consequent to epigenetic modifications on histones, as shown by some histone markers known as super-enhancers [117].

N-RAS mutation has also been studied in zebrafish and its expression led to hyperpigmentation throughout the zebrafish’s body. When p53 mutation was added to mutated N-RAS, the fish developed invasive melanomas which were histologically and genetically correlated to human melanomas.

Although less frequent in melanomas than the previously mentioned BRAF and N-RAS mutation, H-RAS-mutated zebrafish models also displayed melanoma development [90, 118, 119].

Combining these assets with the excellent melanoma models engineered in zebrafish has led to several significant advances in our knowledge of melanoma behavior and molecular asset. New frontiers involve testing even infrequently mutated potential drivers, thus broadening the available models of cutaneous melanoma and introducing non-cutaneous melanoma zebrafish models [120]. Moreover, loss of function CRISPR/Cas9 gene targeting technology has been successfully used to create loss of function models, allowing testing of candidates that may alter disease onset and/or progression.

As an example, this technique was used to investigate SPRED1 function as a tumor suppressor in the context of KIT mutations in mucosal melanoma. SPRED1 knockdown, determining MAPK activation, conferred resistance to drugs inhibiting KIT tyrosine kinase activity. MAPK inhibition in SPRED1-deficient melanomas could therefore be a therapeutic hint and again proves the power of zebrafish modeling to investigate genetic interactions in cancer pathways [121].

Concerning the aforementioned cancer intra-tumoral heterogeneity, single cell RNA sequencing (sc-RNA sq) technologies provide an insight into melanoma complexity [122]. Analysis of cell dynamics at the minimal residual disease (MRD) stage, when persistent cells in otherwise disease-free tissue acquire specific properties for melanoma progression, proves fundamental to grasp the tumor vulnerability at a crucial point [123]. Sc-RNA sq was used to study MITF-low state role in melanoma progression in zebrafish genetic models with low activity of Mitfa, proving that very low or absent MITF activity characterized a residual disease like therapy-resistant melanoma [124]. Additional research on melanoma cells interaction with their microenvironment has been accomplished in a transgenic zebrafish model, proving the power of tools such as spatially resolved transcriptomics, sc- RNA-seq, and single-nucleus RNA-seq [125].

Interaction with metabolism has rarely been considered as an impacting factor in cancer and, more specifically, in melanoma; however, interference with liver gluconeogenesis has been successfully investigated in a zebrafish melanoma model through isotope tracing, confirming versatility of zebrafish in the field of research [126].

Not only has the zebrafish model helped to investigate melanoma genesis and development as far as its genetics is concerned, but also it has recently offered an insight into new therapeutic strategies for melanoma metastatic progression by targeting specific signaling cascades. For instance, human epidermal growth factor receptor (EGFR) signaling was implied when PLD c GMP analog protein kinase G activator 5 (PA5) was injected into zebrafish melanoma models, thus targeting the cGMP/protein kinase G pathway [127]. Another receptor tyrosine kinase, Xrmk, was identified as closely related to EGFR, and therefore involved in melanoma development and progression; in detail, Xrmk has been studied in Xiphophorus platyfish and in zebrafish as a therapeutic target [128]. Moreover, the activation of CD271, a member of the tumor necrosis factor receptor (TNFR) family, using a short β-amyloid-derived peptide, combined with chemotherapy or MAPK inhibitors, proved to significantly reduce metastasis in a zebrafish xenograft model [129].

### 3.2 Squamous cell carcinoma models in zebrafish

Even though non-melanoma skin cancer in fish is less common compared to melanoma, zebrafish have been adequately used as a model to study the underlying pathogenetic mechanisms in these kinds of cancer as well.

Recent works that employ the SCC xenograft model in zebrafish have identified key molecules involved in the pathogenesis of squamous cell carcinoma (SCC) [130], as well as compounds that may be used as targets for SCC therapy [131]. A crucial molecule to be studied as a therapeutic target is the tyrosine kinase receptor Axl, which is highly expressed in SCC [132]. Other important targets are the COL7A1 gene, which is responsible for the development of aggressive SCCs in epidermolysis bullosa, and the recombinant type VII collagen (hrCol7), which is able to reverse SCC angiogenesis in the zebrafish model [133].
Another interesting in vivo xenograft model study has analyzed the role of the tyrosine kinase discoidin domain receptor 2 (DDR2) in cell proliferation, adhesion, differentiation and invasion in head and neck squamous cell carcinoma (HNSCC) [134]. The study shows that dasatinib, a Food and Drug Administration (FDA)-approved inhibitor of c-Kit, Proto-oncogene tyrosine-protein kinase (ABL, SRC) and Abelson murine leukemia viral oncogene homolog, may be potentially used in DDR2-positive SCC patients to block tumor cell invasion and migration [134].

Another potential compound for HNSCC treatment is the marine microbial extract luminacin. Studies in zebrafish embryos have shown that luminacin treatment of tumor cells stimulates autophagy in SCC cell lines, thus inhibiting cancer growth and progression [130].

Lastly, the zebrafish model has also been used to show that Flotillin-1 over-expression in KB cells (a subline of the keratin-forming tumor cell line HeLa) boosts KB cell motility and cell growth [135].

These studies prove that the zebrafish model may be adequately used not only in the evaluation of molecular pathways involved in SCC development and progression, but also in drug toxicity and screening assays.

3.3 Other dermatological applications of zebrafish

Zebrafish can be used to study not only cancer derived from melanocytes, but also other disorders of melanogenesis, since melanogenesis pathways are conserved between zebrafish and mammals, and melanogenesis is a visible process in zebrafish embryos and in transparent casper adults [136]. Studying zebrafish albinism models, researchers could clarify the function of genes whose role in the pathogenesis of this disorder remains concealed and that might not yet be recognized as implicated in human albinism. Correct genetic diagnosis might prove crucial in treatment of different, but often clinically indistinguishable, forms of albinism. Thus, rapid CRISPR screening for gene function makes zebrafish an excellent model for albinism gene discovery. Though counterintuitive, zebrafish albinism models could also help clarify chemotherapeutic resistance mechanisms in cancerous melanocytes in melanoma [137, 138].

Hereditary pigment disorders have been investigated using MOs to ascertain the function of specific genes that had previously been identified in affected individuals. Protein O-fucosyltransferase 1 (pofut1) and presenilin enhancer-2 (psenen) knockdown zebrafish both displayed abnormal distribution in pigmentation, thus confirming involvement of the aforementioned genes in certain clinical presentations of Dowling-Degos syndrome, also known as reticulate pigmented anomaly of flexures. Furthermore, oca2-mutant zebrafish and c10orf11 knockout zebrafish were created to explore oculocutaneous albinism-related gene function in vivo, confirming involved conserved gene function throughout fish, mouse and humans. Hypopigmentation characterized also snow white zebrafish mutant carrying a hps5 gene mutation, reproducing Hermansky-Pudlak syndrome (HPS) in fish models. Zebrafish fade out mutant also recreated HPS phenotype indicating that fade out gene could have a role in the pathogenesis of HPS. Disorders of copper metabolism were also reproduced in zebrafish with the calamity and catastrophe mutant models, underlying the influence that copper and, potentially, other nutrients, could have on melanin synthesis in melanocytes. Impact of stress on vitiligo development in fish was reproduced by treating zebrafish with interleukin-17, which determined altered pigmentation and autophagy in pigment cells [138].

Mutation in NRAS resulting in an I24N amino acid substitution was identified in an individual bearing typical Noonan syndrome features. N-Ras-I24N expressing zebrafish displayed developmental defects which were parallel to other Noonan syndrome-associated genes in zebrafish. Activation in N-RAS signaling pathway was therefore confirmed to be associated to a Noonan Syndrome phenotype. Of note, MEK inhibition completely rescued the activated N-Ras-induced phenotypes, confirming the exclusive mediation of Ras-MAPK signaling in the genesis of the syndrome [139].

Co-occurrence of Mongolian blue spots with vascular birthmarks defines a group of syndromes known as phakomatosis pigmentovascularis. Association with activating mutations in GNA11 and GNAQ genes, encoding a Ga subunit of heterotrimeric G proteins, was discovered and confirmed in a transgenic mosaic zebrafish model expressing mutant GNA11R183C
under *mitfa* promoter, which developed extensive dermal melanocytosis recapitulating the human phenotype. Specifically, zebrafish embryos were injected with wild type human GNA11, GNA11R183C, or GNA11Q209L expressed under control of the melanocyte *mitfa* promoter. The embryos were grown to adult fish; their status of genetically mosaic animals was clinically visible as melanocyte patches, which received histological confirmation [140].

RASopathies result from germline mutations of the Ras/MAPK pathway. Systematic predictions on disease progression are not yet possible, even though available technologies in genome sequencing allow to identify multiple disease-related mutations. Nevertheless, zebrafish embryos represent a valuable model in assessing mutational effects. Jindal et al. succeeded in ranking several MEK1 mutations, proving that those found in cancer were more severe than those found in shared by RASopathies and cancer. Also, the latter resulted as more severe than those characterizing only RASopathies. A conserved ranking was observed in Drosophila and the ranking could predict the drug dose to correct the defects [141]. Wound healing and re-epithelialization of adult zebrafish skin have been analyzed in several studies. In zebrafish the process of wound healing results in minimal scar formation. The process comprises a series of events: rapid re-epithelialization; migration of inflammatory cells; formation of granulation tissue consisting of macrophages, fibroblasts, blood vessels, and collagen; granulation tissue regression. Major steps and principles of cutaneous wound healing seem to be the same in adult mammals and adult zebrafish, thus making the zebrafish a valuable model for studying vertebrate skin repair [142]. Richardson et al. studied the wound healing process by creating full-thickness wounds with a laser on the flank of adult zebrafish in a rapid and reproducible way, confirming that the zebrafish is a unique and cost-effective model for skin repair [142]. Absence of wound scars in zebrafish, as observed in human embryos, due to the lack of the blood-clotting phase and to specific signaling mechanism, represents an attractive model to study healing processes and is expected to help to formulate an appropriate drug for cutaneous wound healing [143].
| Tumor type/cutaneous condition | Involved gene/pathway or study drug | Technique | References |
|--------------------------------|------------------------------------|-----------|------------|
| Oral squamous cell carcinoma  | Sandensolide at different concentrations | Human cancer xenograft in 48 hpf embryos; incubation with different sandensolide concentrations | Yu et al. [153] |
| Epidermal adhesion mechanism deficit (Kindler syndrome, KS) | Kindlin-1 (regulator of integrin function) | Morpholino oligomers (MOs); stably mutated fish line | Postel et al. [154] |
| Epidermal stratification; keratinocyte proliferation and differentiation | TAp63 and p53 | Target-selected mutagenesis (TILLING) and TEAZ (transgene electroporation in adult zebrafish) | Fischer et al. [22] |
| Psoriasis                      | Homozygous m14 mutant              | EMS-induced mutagenesis; whole-mount immunohistochemistry | Webb et al. [147] |
| Cytokine function              | IL-1β, TNF-α, IL-6, IL-2, IFN-γ, IL-4/13, IL-10, IL-17 A/F, IL-21, IL-22, TGF-β1, IFN and TNF-α | Knock-down with morpholinos and overexpression of G-CSF by injection of eggs with in vitro transcribed cytokines mRNA | Zou et al. [39] |
| Melanoma                       | Nodal signaling                    | Human cancer xenograft transplantation models in zebrafish blastula 3hpf | Topczewska et al. [155] |
| Melanoma                       | BRAFV600E tp53M214K                 | Genetic model (BRAF/p53) | Patton et al. [85] |
| Melanoma                       | BRAFV600E tp53M214K; crestin:EGFP  | Genetic model (BRAF/p53, crestin gene- Reactivation), Crestin-based reporter transgene | Kaufman et al. [86] |
| Melanoma                       | BRAFV600E mitfavc7                  | Genetic model (BRAF plus conditional MITF mutation) | Lister et al. [87] |
| Melanoma                       | Oncogenic HRAS under the kita promoter; RAS-induced micro-RNAs (miR-146a and 193a) targeting Jmjd6 | Genetic model (injection of fertilized eggs with plasmids) | Santoriello et al. [90] |
| Melanoma                       | SETDB1                             | Eupigenetic regulators in zebrafish cancer; miniCoopR vector system | Ceol et al. [156] |
| Melanoma                       | BRAF, tp53                          | Cancer allograft transplantation model in rag2E450fs, jak3P369fs, and prkdC 361 2fs mutant fish. Immuno compromised transparent (casper) adult fish (primary cells) | Tang et al. [157, 158] |
| Melanoma                       | BRAFV600E, tp53/−/−                 | CRISPR technologies to modify zebrafish melanoma cell lines and the casper recipient. Cancer allograft transplantation model in transparent (casper) adult fish and in 48-hpf embryos | Heilmann et al. [160] |
| Melanoma                       | BRAFV600E, tp53/−/−                 | Cancer allograft transplantation model in transparent (casper) fish | Benjamin et al. [161] |
| Melanoma                       | BRAFV600E, tp53/−/−                 | Tracking of zebrafish melanoma extracellular vesicles | Hyenne et al. [162] |
| Melanoma                       | BRAF, KRAS                          | Human cancer xenograft transplantation models in zebrafish blastula | Lee et al. [163] |
| Melanoma                       | BRAF, PTEN                          | Human cancer xenograft transplantation in 2-dpf embryos | Haldi et al. [164] |
| Melanoma                       | BRAF, p53, ras-MAPK pathways        | Murine cancer xenograft transplantation models in 48-hpf embryos | Nicoli et al. [165] |
| Melanoma                       | BRAFV600E                           | Human patient-derived cancer xenograft transplantation (PDX) models in two-month-old immunocompromised zebrafish | Yan et al. [167] |
The aforementioned similarities of the zebrafish integument structure together with those of the inflammation mechanisms, make this teleost a fundamental and cost-effective model also to study major dermatologic inflammatory diseases, such as psoriasis. Several models, including mutant, morphant and environmentally inducible models, were created to investigate genetic alterations and molecular mechanisms of psoriasis [144–152].

4 Conclusions

Inflammatory and neoplastic skin disorders are very common and are increasing worldwide.

Zebrafish can provide a suitable animal model to extend our understanding of the molecular and cellular mechanisms of skin disorders and to develop new therapeutic strategies in dermatology (Fig. 2). Zebrafish models of major interest in dermatological research are summarized in Table 1 [22, 39, 85–87, 89, 147, 153–167].

Owing to its low maintenance cost, highly conserved genome, and easy genetic manipulation, the zebrafish is an excellent model for preclinical research in dermatological laboratories, thus bridging the gap between in vitro cell culture an in vivo mammalian models.

5 Methodological approach

The database of Pubmed was queried with the following search string (zebrafish OR/AND dermatology* OR skin cancer* OR melanoma*) under all fields (last search December 2021).

Acknowledgements NT was supported by AIRC (Grant IG-2017-19928), Italian Telethon (Grant GGP19287), IOV 5 x 1000 (Gant METAMELAHP-NAP), and “Piccoli Punti” foundation.

Author contributions IR, ES, LF and NT wrote the main manuscript text and NT prepared figures. All authors reviewed the manuscript. All authors read and approved the final manuscript.

Declarations

Competing interests The authors declare no competing interests.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

1. Sarasamma S, Lai YH, Liang ST, Liu K, Hsiao CD. The power of fish models to elucidate skin cancer pathogenesis and impact the discovery of new therapeutic opportunities. Int J Mol Sci. 2018;19(12):3929. https://doi.org/10.3390/ijms19123929.
2. Schartl M, Walter RB. Xiphophorus and medaka cancer models. Adv Exp Med Biol. 2016;916:531–52. https://doi.org/10.1007/978-3-319-30654-4_23.
3. Hyodo-Taguchi Y, Matsudaira H. Induction of transplantable melanoma by treatment with N-methyl-N′-nitro-N-nitrosoguanidine in an inbred strain of the teleost Oryzias latipes. J Natl Cancer Inst. 1984;73(5):1219–27.
4. Streisinger G, Walker C, Dower N, Knauber D, Singer F. Production of clones of homozygous diploid zebra fish (Brachydanio rerio). Nature. 1981;291(5813):293–6. https://doi.org/10.1038/291293a0.
5. Grunwald DJ, Streisinger G. Induction of recessive lethal and specific locus mutations in the zebrafish with ethyl nitrosourea. Genet Res. 1992;59(2):103–16. https://doi.org/10.1017/s0016672300030317.
6. Haffter P, Granato M, Brand M, et al. The identification of genes with unique and essential functions in the development of the zebrafish, Danio rerio. Development. 1996;123:1–36.
7. Postlethwait JH, Yan YL, Gates MA, et al. Vertebrate genome evolution and the zebrafish gene map. Nat Genet. 1998;18(4):345–9. https://doi.org/10.1038/ng0498-345 (published correction appears in Nat Genet 1998 Jul;19(3):303).
8. Howe K, Clark MD, Torroja CF, et al. The zebrafish reference genome sequence and its relationship to the human genome. Nature. 2013;496(7446):498–503. https://doi.org/10.1038/nature12111.
9. Bootorabi F, Manouchehri H, Changizi R, et al. Zebrafish as a model organism for the development of drugs for skin cancer. Int J Mol Sci. 2017;18(7):1550. https://doi.org/10.3390/ijms18071550.
10. Hason M, Bartůněk P. Zebrafish models of cancer-new insights on modeling human cancer in a non-mammalian vertebrate. Genes. 2019;10(11):935. https://doi.org/10.3390/genes10110935.

11. White RM, Sessa A, Burke C, et al. Transparent adult zebrafish as a tool for in vivo transplantation analysis. Cell Stem Cell. 2008;2(2):183–9. https://doi.org/10.1016/j.stem.2007.11.002.

12. Dahm R, Geisler R. Learning from small fry: the zebrafish as a genetic model organism for aquaculture fish species. Mar Biotechnol. 2006;8(4):329–45. https://doi.org/10.1016/s1066-5139-0. 2003.

13. Verkman AS. Drug discovery in academia. Am J Physiol Cell Physiol. 2004;286(3):C465–74. https://doi.org/10.1152/ajpcell.00397. 2003.

14. Veldman MB, Lin S. Zebrafish as a developmental model organism for pediatric research. Pediatr Res. 2008;64(5):470–6. https://doi.org/10.1203/PDR.0b013e31818ee609.

15. Fernandez Del Ama L, Jones M, Walker P, Chapman A, Braun JA, Mohr J, Hurlstone AF. Reprofiling using a zebrafish melanoma model reveals drugs cooperating with targeted therapeutics. Oncotarget. 2016;7(26):40348–61. https://doi.org/10.18632/oncotarget.9613.

16. Spence R, Gerlach G, Lawrence C, Smith C. The behaviour and ecology of the zebrafish, Danio rerio. Biol Rev Camb Philos Soc. 2008;83(1):13–34. https://doi.org/10.1111/j.1469-185X.2007.00030.x.

17. Force A, Lynch M, Pickett FB, Amores A, Yan YL. Postlethwait J. Preservation of duplicate genes by complementary, degenerative mutations. Genetics. 1999;151(4):1531–45. https://doi.org/10.1093/genetics/151.4.1531.

18. Taylor JS, Braasch I, Frickey T, Meyer A, Van de Peer Y. Genome duplication, a trait shared by 22000 species of ray-finned fish. Genome Res. 2003;13(3):382–90. https://doi.org/10.1101/gr.4640303.

19. Staudt N, Müller-Siersneth N, Fane-Dremucheva A, Yusaf SP, Millrine D, Wright GJ. A panel of recombinant monoclonal antibodies against zebrafish neural receptors and secreted proteins suitable for wholemount immunostaining. Biochem Biophys Res Commun. 2015;456(1):527–33. https://doi.org/10.1016/j.bbrc.2014.11.123.

20. Traver D, Paw BH, Poss KD, Penberthy WT, Lin S, Zon LL. Transplantation and in vivo imaging of multilineage engrafment in zebrafish bloodless mutants. Nat Immunol. 2003;4(12):1238–46. https://doi.org/10.1038/nili007.

21. Hawkins JW. The structure of fish skin I. General organization. Cell Tissue Res. 1974;149(2):147–58. https://doi.org/10.1006/cimm.1994.1154.

22. Fischer B, Metzger M, Richardson R, et al. P53 and TAp63 promote keratinocyte proliferation and differentiation in breeding tubercles of the zebrafish. PLoS Genet. 2014;10(1):e1004048. https://doi.org/10.1371/journal.pgen.1004048.

23. Henrikson RC, Matoltsy AG. The fine structure of teleost epidermis. 1. Introduction and filament-containing cells. J Ultrastruct Res. 1967;21(3):194–212. https://doi.org/10.1016/s0022-5320(67)80081-1.

24. Chang WJ, Hwang PP. Development of zebrafish epidermis. Birth Defects Res C Embryo Today. 2011;93(3):205–14. https://doi.org/10.1002/bdrc.20215.

25. Li Q, Uitto J. Zebrafish as a model system to study skin biology and pathology. J Invest Dermatol. 2014;134(6):1–6. https://doi.org/10.1038/jid.2014.182.

26. Ni-Komatsu L, Orlow SJ. Identification of novel pigment modulators by chemical genetic screening. J Invest Dermatol. 2007;127(7):1585–92. https://doi.org/10.3831/jjd.5700852.

27. Budi EH, Patterson LB, Parichy DM. Post-embryonic nerve-associated precursors to adult pigment cells: genetic requirements and dynamics of morphogenesis and differentiation. PLoS Genet. 2011;7(5):e1002044. https://doi.org/10.1371/journal.pgen.1002044.

28. Rawls JF, Johnson SL. Temporal and molecular separation of the kit receptor tyrosine kinase’s roles in zebrafish melanocyte migration and survival. Dev Biol. 2003;262(1):152–61. https://doi.org/10.1016/s0012-1606(03)00386-5.

29. Hultman KA, Johnson SL. Differential contribution of direct-developing and stem cell-derived melanocytes to the zebrafish larval pigment pattern. Dev Biol. 2010;337(2):425–31. https://doi.org/10.1016/j.ydbio.2009.11.019.

30. Delamare-Deboutville J, Wood D, Barnes AC. Response and function of cutaneous mucosal and serum antibodies in barramundi (Lates calcarifer) acclimated in seawater and freshwater. Fish Shellfish Immunol. 2006;21(1):92–101. https://doi.org/10.1016/j.fsi.2005.10.005.

31. Al-Soudi A, Kaaij MH, Tas SW. Endothelial cells: from innocent bystanders to active participants in immune responses. Autoimmun Rev. 2017;16(9):951–62. https://doi.org/10.1016/j.autrev.2017.07.008.

32. Harris RA. Spatial, temporal, and functional aspects of macrophages during “the good, the bad, and the ugly” phases of inflammation. Front Immunol. 2014;5:612. https://doi.org/10.3389/fimmu.2014.00612.

33. Ni-Komatsu L, Orlow SJ. Identification of novel pigment modulators by chemical genetic screening. J Invest Dermatol. 2007;127(7):1585–92. https://doi.org/10.3831/jjd.5700852.

34. Budi EH, Patterson LB, Parichy DM. Post-embryonic nerve-associated precursors to adult pigment cells: genetic requirements and dynamics of morphogenesis and differentiation. PLoS Genet. 2011;7(5):e1002044. https://doi.org/10.1371/journal.pgen.1002044.

35. Hultman KA, Johnson SL. Differential contribution of direct-developing and stem cell-derived melanocytes to the zebrafish larval pigment pattern. Dev Biol. 2010;337(2):425–31. https://doi.org/10.1016/j.ydbio.2009.11.019.

36. Delamare-Deboutville J, Wood D, Barnes AC. Response and function of cutaneous mucosal and serum antibodies in barramundi (Lates calcarifer) acclimated in seawater and freshwater. Fish Shellfish Immunol. 2006;21(1):92–101. https://doi.org/10.1016/j.fsi.2005.10.005.

37. Al-Soudi A, Kaaij MH, Tas SW. Endothelial cells: from innocent bystanders to active participants in immune responses. Autoimmun Rev. 2017;16(9):951–62. https://doi.org/10.1016/j.autrev.2017.07.008.

38. Harris RA. Spatial, temporal, and functional aspects of macrophages during “the good, the bad, and the ugly” phases of inflammation. Front Immunol. 2014;5:612. https://doi.org/10.3389/fimmu.2014.00612.

39. Ni-Komatsu L, Orlow SJ. Identification of novel pigment modulators by chemical genetic screening. J Invest Dermatol. 2007;127(7):1585–92. https://doi.org/10.3831/jjd.5700852.

40. Budi EH, Patterson LB, Parichy DM. Post-embryonic nerve-associated precursors to adult pigment cells: genetic requirements and dynamics of morphogenesis and differentiation. PLoS Genet. 2011;7(5):e1002044. https://doi.org/10.1371/journal.pgen.1002044.
43. Sommer F, Torraca V, Meijer AH. Chemokine receptors and phagocyte biology in zebrafish. Front Immunol. 2020;11:325. https://doi.org/10.3389/fimmu.2020.00325.

44. Meseguer J, López-Ruiz A, Angeles Esteban M. Cytochemical characterization of leucocytes from the seawater teleost, gilthead seabream (Sparus aurata L.). Histochemistry. 1994;102(1):37–44. https://doi.org/10.1007/BF00271047.

45. Morcillo P, Cordero H, Meseguer J, Esteban M, Cuesta A. Toxicological in vitro effects of heavy metals on gilthead seabream (Sparus aurata L.) head-kidney leucocytes. Toxicol In Vitro. 2015;30(1 Pt B):412–20. https://doi.org/10.1016/j.tiv.2015.09.021.

46. Sepulcre MP, Pelegrín P, Mulero V, Meseguer J. Characterization of gilthead seabream acidophilic granulocytes by a monoclonal antibody unequivocally points to their involvement in fish phagocytic response. Cell Tissue Res. 2002;308(1):97–102. https://doi.org/10.1007/s00441-002-0531-1.

47. Burhans MS, Hageman DK, Kuzma JN, Meschedt KA, Kratz M. Contribution of adipose tissue inflammation to the development of type 2 diabetes mellitus. Compr Physiol. 2018;9(1):1–58. https://doi.org/10.1002/cphy.c170040.

48. Imitola J, Raddassi K, Park KI, et al. Directed migration of neural stem cells to sites of CNS injury by the stromal cell-derived factor 1alpha/CXC chemokine receptor 4 pathway. Proc Natl Acad Sci USA. 2004;101(52):18117–22. https://doi.org/10.1073/pnas.0408258102.

49. Block ML, Zecca L, Hong JS. Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. Nat Rev Neurosci. 2007;8(1):57–69. https://doi.org/10.1038/nrn2038.

50. Medzhitov R. Origin and physiological roles of inflammation. Nature. 2008;454(7203):428–35. https://doi.org/10.1038/nature07201.

51. Nathan C. Neutrophils and immunity: challenges and opportunities. Nat Rev Immunol. 2006;6(3):173–82. https://doi.org/10.1038/nri1785.

52. Nguyen-Chi M, Laplace-Builhe B, Travnickova J, et al. Identification of polarized macrophage subsets in zebrafish. Elife. 2015;4:e07288. https://doi.org/10.7554/eLife.07288.

53. Korkmaz B, Horwitz MS, Jenne DE, Gauthier F. Neutrophil elastase, proteinase 3, and cathepsin G as therapeutic targets in human diseases. Pharmacol Rev. 2010;62(4):726–59. https://doi.org/10.1177/0034674809350577.

54. Larsen GL, Henson PM. Mediators of inflammation. Annu Rev Immunol. 1983;1:335–59. https://doi.org/10.1146/annurev.iy.01.040183.002003.

55. Chaves-Pozo E, Muñoz P, López-Muñoz P, Pelegrín P, García Ayala A, Mulero V, Meseguer J. Early innate immune response and redistribution of inflammatory cells in the bony fish gilthead seabream experimentally infected with Vibrio anguillarum. Cell Tissue Res. 2005;320(1):61–8. https://doi.org/10.1007/s00441-004-1063-7.

56. Press CM, Evesen O. The morphology of the immune system in teleost fishes. Fish Shellfish Immunol. 1999;9(4):309–18. https://doi.org/10.1016/S0892-6236(99)00181-X.

57. Gonzalez TJ, Lu Y, Boswell M, et al. Fluorescent light exposure incites acute and prolonged immune responses in zebrafish (Danio rerio) skin. Comp Biochem Physiol C Toxicol Pharmacol. 2018;208:87–95. https://doi.org/10.1016/j.cbpc.2017.09.009.

58. Mroz EA, Raddassi K, Roswell M, et al. Inflammation in zebrafish. Lab Invest. 2000;80(3):379–85. https://doi.org/10.1038/labinvest.3780042.

59. Block ML, Zecca L, Hong JS. Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. Nat Rev Neurosci. 2007;8(1):57–69. https://doi.org/10.1038/nrn2038.

60. Cagan RL, Zon LI, White RM. Modeling cancer with flies and fish. Dev Cell. 2019;49(3):317–24. https://doi.org/10.1016/j.devcel.2019.04.012.

61. Grzywa TM, Paskal W, Włodarski PK. Intratumor and intertumor heterogeneity in melanoma. Transl Oncol. 2017;10(6):956–75. https://doi.org/10.1016/j.tranon.2017.09.007.

62. Cagan RL, Zon LI, White RM. Modeling cancer with flies and fish. Dev Cell. 2019;49(3):317–24. https://doi.org/10.1016/j.devcel.2019.04.013.

63. McCune JM, Namikawa R, Kaneshima H, Shultz LD, Lieberman M, Weissman IL. The SCID-hu mouse: murine model for the analysis of human hematolymphoid differentiation and function. Science. 1988;241(4873):1632–9. https://doi.org/10.1126/science.241.4873.1632.

64. Böck BC, Stein U, Schmitt CA, Augustin HG. Mouse models of human cancer. Cancer Res. 2014;74(17):4671–5. https://doi.org/10.1158/0008-5472.CAN-14-1424.

65. Capasso A, Lang J, Pitts TM, et al. Characterization of immune responses to anti-PD-1 mono and combination immunotherapy in hematopoietic humanized mice implanted with tumor xenografts. J Immunother Cancer. 2019;7(1):37. https://doi.org/10.1186/s40425-019-0512-8.

66. Amatruda JF, Shepard JL, Stern HM, Zon LI. Zebrafish as a cancer model system. Cancer Cell. 2002;1(3):229–31. https://doi.org/10.1016/S1538-4107(02)00052-1.

67. Beckwith LG, Moore JL, Tsao-Wu GS, Harshbarger JC, Cheng KC. EthylNitrosourea induces neoplasia in zebrafish (Danio rerio). Lab Invest. 2000;80(3):379–85. https://doi.org/10.1038/labinvest.3780042.

68. Spitsbergen JM, Tsai HW, Reddy A, Miller T, Arboqat D, Hendricks JD, Bailey GS. Neoplasia in zebrafish (Danio rerio) treated with N-methyl-N′-nitro-N-nitrosoguanidine by three exposure routes at different developmental stages. Toxicol Pathol. 2000;28(5):716–25. https://doi.org/10.1080/019262330002800512.

69. Sassen WA, Köster R. A molecular toolbox for genetic manipulation of zebrafish. Adv Genom Genet. 2015;5:151–63. https://doi.org/10.2147/AGG.S57585.

70. Nasevicius A, Ekker SC. Effective targeted gene ‘knockdown’ in zebrafish. Nat Genet. 2000;26(2):216–20. https://doi.org/10.1038/79951.

71. Dougherty MC, Kamm MC, McCammon JM, Miller JC, et al. Heritable targeted gene disruption in zebrafish using designed zinc-finger nucleases. Nat Biotechnol. 2008;26(6):702–8. https://doi.org/10.1038/nbt.1409.

72. Huang P, Xiao A, Zhou M, Zhu Z, Lin S, Zhang B. Heritable targeted gene targeting in zebrafish using customized TALENs. Nat Biotechnol. 2011;29(8):699–700. https://doi.org/10.1038/nbt.1939.

73. Ablain J, Durand EM, Yang S, Zhou Y, Zon LI. A CRISPR/Cas9 vector system for tissue-specific gene disruption in zebrafish. Dev Cell. 2015;32(6):756–64. https://doi.org/10.1016/j.devcel.2015.01.032.

74. Kok FQ, Shin M, Ni CW, et al. Reverse genetic screening reveals poor correlation between morpholino-induced and mutant phenotypes in zebrafish. Dev Cell. 2015;32(1):97–108. https://doi.org/10.1016/j.devcel.2014.11.018.

75. Stainier DY, Kontarakis Z, Rossi A. Making sense of anti-sense data. Dev Cell. 2015;32(1):7–8. https://doi.org/10.1016/j.devcel.2014.12.012.

76. Yin L, Maddison LA, Chen W. Multiplex conditional mutagenesis in zebrafish using the CRISPR/Cas system. Methods Cell Biol. 2016;135:3–17. https://doi.org/10.1016/bcm.2016.04.018.
75. Liu K, Petree C, Requena T, Varshney P, Varshney GK. Expanding the CRISPR toolbox in zebrafish for studying development and disease. Front Cell Dev Biol. 2019;7:13. https://doi.org/10.3389/fcell.2019.00013.

76. Callahan SJ, Tepan S, Zhang YM, et al. Cancer modeling by transgene electroporation in adult zebrafish (TEAZ). Dis Model Mech. 2018;11(9):dm034561. https://doi.org/10.1242/dmm.034561.

77. Kirchberger S, Sturtzel C, Pascoal S, Distel M. Quo natas, Dania?—recent progress in modeling cancer in zebrafish. Front Oncol. 2017;7:186. https://doi.org/10.3389/fonc.2017.00186.

78. Brown HK, Schiavone K, Tazzymans, et al. Zebrafish xenograft models of cancer and metastasis for drug discovery. Expert Opin Drug Discov. 2017;12(4):379–89. https://doi.org/10.1080/17460441.2017.1297416.

79. Lin J, Zhang W, Zhao J, et al. A clinically relevant in vivo zebrafish model of human multiple myeloma to study preclinical therapeutic efficacy. Blood. 2016;128(2):249–52. https://doi.org/10.1182/blood-2016-03-704660.

80. Berghmans S, Murphy RD, Wiemholds E, et al. tp53 mutant zebrafish develop malignant peripheral nerve sheath tumors. Proc Natl Acad Sci USA. 2005;102(2):407–12. https://doi.org/10.1073/pnas.0406252102.

81. Ignatius MS, Hayes MN, Moore FE, et al. tp53 deficiency causes a wide tumor spectrum and increases embryonal rhabdomyosarcoma metastasis in zebrafish. Elife. 2018;7:e37202. https://doi.org/10.7554/elife.37202.

82. Mensah L, Ferguson JL, Shive HR. Genotypic and phenotypic variables affect meiotic cell cycle progression, tumor ploidy, and cancer-associated mortality in a brca2-mutant zebrafish model. J Oncol. 2019;2019:9218251. https://doi.org/10.1155/2019/9218251.

83. Langenau DM, Keefe MD, Storer NY, et al. Co-injection strategies to modify radiation sensitivity and tumor initiation in transgenic zebrafish. Oncogene. 2008;27(30):4242–8. https://doi.org/10.1038/onc.2008.56.

84. Langenau DM, Keefe MD, Storer NY, et al. Effects of RAS on the genesis of embryonal rhabdomyosarcoma. Genes Dev. 2007;21(11):1382–95. https://doi.org/10.1101/gad.1545007.

85. Santoriello C, Deflorian G, Pezzimenti F, Kawakami K, Lanfrancone L, di d'Adda F, Mione FM. Expression of H-RASV12 in a zebrafish model-driven hepatocellular carcinoma (HCC) model. Dis Model Mech. 2019;12(2):dmm038240. https://doi.org/10.1242/dmm.038240.

86. Lister JA, Capper A, Zeng Z, et al. A conditional zebrafish MITF mutation reveals MITF levels are critical for melanoma promotion vs. regression in vivo. J Invest Dermatol. 2014;134(1):133–40. https://doi.org/10.1038/jid.2013.293.

87. Santoriello C, Chhangawala S, et al. Oncogenic BRAF disrupts thyroid morphogenesis and function via twist expression. Elife. 2017;6:e20728. https://doi.org/10.7554/eLife.20728.

88. Kirchberger S, Scocca S, Distel M. Brca2-mutant zebrafish model. J Oncol. 2019;2019:9218251. https://doi.org/10.1155/2019/9218251.

89. Chou YT, Chen LY, Tsai SL, et al. Ribose-5-phosphate isomerase A overexpression promotes liver cancer development in transgenic zebrafish. Oncogene. 2008;27(30):4242–8. https://doi.org/10.1038/onc.2008.56.

90. Santoriello C, Gennaro E, Anelli V, et al. Kita driven expression of oncogenic HRAS leads to early onset and highly penetrant melanoma in zebrafish. PLoS ONE. 2010;5(12):e15170. https://doi.org/10.1371/journal.pone.0015170.

91. Anelli V, Villefranc JA, Chhangawala S, et al. Oncogenic BRAF disrupts thyroid morphogenesis and function via twist expression. Elife. 2017;6:e20728. https://doi.org/10.7554/eLife.20728.

92. Park JT, Leach SD. Zebrafish model of KRAS-induced progenitor cell expansion and malignant transformation in zebrafish exocrine pancreas. Gastroenterology. 2008;134(7):2080–90. https://doi.org/10.1053/j.gastro.2008.02.084.

93. Park JT, Leach SD. Zebrafish model of KRAS-initiated pancreatic cancer. Anim Cell Syst. 2018;22(6):353–9. https://doi.org/10.1007/19768354.2018.1530321.

94. Chou YT, Chen LY, Tsai SL, et al. Ribose-5-phosphate isomerase A overexpression promotes liver cancer development in transgenic zebrafish via activation of ERK and β-catenin pathways. Carcinogenesis. 2019;40(3):461–73. https://doi.org/10.1093/carcin/bgy155.

95. Yeh JR, Munson KM, Chao YL, et al. Oncogenic KRAS induces progenitor cell expansion and malignant transformation in zebrafish exocrine pancreas. Transl Res. 2014;163(5):429–39. https://doi.org/10.1016/j.trsl.2014.02.006.

96. Shive HR, West RR, Embree L, et al. Oncogenic BRAF disrupts thyroid morphogenesis and function via twist expression. Elife. 2017;6:e20728. https://doi.org/10.7554/eLife.20728.

97. Santoriello C, Chhangawala S, et al. Oncogenic BRAF disrupts thyroid morphogenesis and function via twist expression. Elife. 2017;6:e20728. https://doi.org/10.7554/eLife.20728.

98. Park SW, Davison JM, Rhee J, Hruban RH, Maitra A, Leach SD. Oncogenic KRAS induces progenitor cell expansion and malignant transformation in zebrafish exocrine pancreas. Gastroenterology. 2008;134(7):2080–90. https://doi.org/10.1053/j.gastro.2008.02.084.

99. Shive HR, West RR, Embree L, et al. Oncogenic BRAF disrupts thyroid morphogenesis and function via twist expression. Elife. 2017;6:e20728. https://doi.org/10.7554/eLife.20728.

100. Santoriello C, Gennaro E, Anelli V, et al. Kita driven expression of oncogenic HRAS leads to early onset and highly penetrant melanoma in zebrafish. PLoS ONE. 2010;5(12):e15170. https://doi.org/10.1371/journal.pone.0015170.

101. Santoriello C, Gennaro E, Anelli V, et al. Oncogenic BRAF disrupts thyroid morphogenesis and function via twist expression. Elife. 2017;6:e20728. https://doi.org/10.7554/eLife.20728.

102. Onnebo SM, Rasighaemi P, Kumar J, Liongue C, Ward AC. Alternative TEL-JAK2 fusions associated with T-cell acute lymphoblastic leukemia. Blood. 2005;106(15):4917–21. https://doi.org/10.1182/blood-2004-09-3294.

103. Langenau DM, Feng H, Kanki JP, et al. Cre/lox-regulated transgenic zebrafish. Gen Comp Endocrinol. 2013;191(3):429–39. https://doi.org/10.1016/j.ygcen.2013.08.004.

104. Santoriello C, Gennaro E, Anelli V, et al. Oncogenic BRAF disrupts thyroid morphogenesis and function via twist expression. Elife. 2017;6:e20728. https://doi.org/10.7554/eLife.20728.

105. Santoriello C, Gennaro E, Anelli V, et al. Oncogenic BRAF disrupts thyroid morphogenesis and function via twist expression. Elife. 2017;6:e20728. https://doi.org/10.7554/eLife.20728.

106. He BL, Shi X, Man CH, et al. Functions of flt3 in zebrafish hematopoiesis and its relevance to human acute myeloid leukemia. Blood. 2014;123(16):2518–29. https://doi.org/10.1182/blood-2013-02-486688.
107. Onnebo SM, Condon MM, McPhee DO, Lieschke GJ, Ward AC. Hematopoietic perturbation in zebrafish expressing a tel-jak2a fusion. Exp Hematol. 2005;33(2):182–8. https://doi.org/10.1016/j.exphem.2004.10.019.

108. Gjini E, Mansour MR, Sander JD, et al. A zebrafish model of myelodysplastic syndrome produced through tet2 genomic editing. Mol Cell Biol. 2015;35(5):789–904. https://doi.org/10.1128/MCB.00971-14.

109. Jin Y, Wei L, Jiang Q, et al. Comparison of efficacy and toxicity of bevacizumab, endostar and apatinib in transgenic and human lung cancer xenograft zebrafish model. Sci Rep. 2018;8(1):15837. https://doi.org/10.1038/s41598-018-34300-5.

110. Chou HL, Lin YH, Liu W, et al. Combination therapy of chloroquine and C2-ceramide enhances cytotoxicity in lung cancer H460 and H1299 cells. Cancers. 2019;11(3):370. https://doi.org/10.3390/cancers11030370.

111. van der Ent W, Johchensen AG, Teunisse AF, et al. Ewing sarcoma inhibition by disruption of EWSR1-FLI1 transcriptional activity and reactivation of p53. J Pathol. 2014;233(4):415–24. https://doi.org/10.1002/path.4378.

112. Buscà R, Baldotti R. Cyclic AMP a key messenger in the regulation of skin pigmentation. Pigment Cell Res. 2000;13(2):60–9. https://doi.org/10.1034/j.1600-0749.2000.130203.x.

113. Chen CH, Lin DS, Cheng CW, et al. Cdc6 cooperates with c-Myc to promote genome instability and epithelial to mesenchymal transition EMT in zebrafish. Oncotarget. 2014;5(15):6300–11. https://doi.org/10.18632/oncotarget.2204.

114. Ekedahl H, Cirenajwis H, Harbst K, et al. The clinical significance of BRAF and NRAS mutations in a clinic-based metastatic melanoma patient cohort. J Invest Dermatol. 2006;126(1):15–60. https://doi.org/10.1038/sj.jid.5700026.

115. Santoriello C, Anelli V, Alghisi E, Mione M. Highly penetrant melanoma in a zebrafish model is independent of ErbB3b signaling. Pigment Cell Melanoma Res. 2012;25(2):287–9. https://doi.org/10.1111/j.1755-148X.2012.00973.x.

116. Ekedahl H, Cirenajwis H, Harbst K, et al. The clinical significance of BRAF and NRAS mutations in a clinic-based metastatic melanoma patient cohort. J Invest Dermatol. 2006;126(1):15–60. https://doi.org/10.1038/sj.jid.5700026.

117. Parichy DM, Rawls JF, Pratt SJ, Whitfield TT, Johnson SL. Zebrafish sparse corresponds to an orthologue of c-kit and is required for the morphogenesis of a subpopulation of melanocytes, but is not essential for hematopoiesis or primordial germ cell development. Development. 1999;162(15):3425–36.

118. Santorini C, Anelli V, Alghisi E, Mione M. Highly penetrant melanoma in a zebrafish model is independent of ErbB3b signaling. Pigment Cell Melanoma Res. 2012;25(2):287–9. https://doi.org/10.1111/j.1755-148X.2012.00973.x.

119. Parichy DM, Rawls JF, Pratt SJ, Whitfield TT, Johnson SL. Zebrafish sparse corresponds to an orthologue of c-kit and is required for the morphogenesis of a subpopulation of melanocytes, but is not essential for hematopoiesis or primordial germ cell development. Development. 1999;162(15):3425–36.

120. Ablain J, Xu M, Rothschild H, et al. Human tumor genomics and zebrafish modeling identify SPRED1 loss as a driver of mucosal melanoma. Science. 2018;362(6418):1055–60. https://doi.org/10.1126/science.aau6509.

121. Rambow F, Rogiers A, Marin-Bejar O, et al. Toward minimal residual disease-directed therapy in melanoma. Cell. 2018;174(4):843–55. https://doi.org/10.1016/j.cell.2018.06.011.

122. Travnickova J, Patton EE. Deciphering melanoma cell states and plasticity with zebrafish models. J Invest Dermatol. 2021;141(6):1389–1402. https://doi.org/10.1016/j.jid.2021.06.006.

123. Wood JS, Morgan WT, Kavathas PJ, et al. Novel use of zebrafish as a model to study the development and progression of melanoma. J Invest Dermatol. 2015;135(8):2089–98. https://doi.org/10.1038/jid.2015.94.

124. Hunter MV, Moncada R, Weiss JM, Yanai I, White RM. Spatially resolved transcriptomics reveals the architecture of the tumor-microenvironment interface. Nat Commun. 2021;12(1):6278. https://doi.org/10.1038/s41467-021-26614-z.

125. Shin YS, Cha HY, Lee BS, et al. Anti-cancer effect of luminacin, a marine microbial extract, in head and neck squamous cell carcinoma xenograft zebrafish model. Sci Rep. 2018;8:15837. https://doi.org/10.1038/s41598-018-34300-5.

126. Monroe JD, Basheer F, Gibert Y. Xmrks the spot: fish models for investigating epidermal growth factor receptor signaling in cancer research. Cells. 2021;10(5):1132. https://doi.org/10.3390/cells10051132.

127. Saltari A, Dzung A, Quadri M, et al. Specific activation of the CD271 intracellular domain in combination with chemotherapy or targeted therapy inhibits melanoma progression. Cancer Res. 2021. https://doi.org/10.1158/0008-5472.CAN-21-0117 (published online ahead of print, 2021 Oct 13).

128. Martins VL, Caley MP, Moore K, et al. Suppression of TGFβ and angiogenesis by type VII collagen in cutaneous squamous cell carcinoma progression via autophagic cell death. Cancer Res Treat. 2016;48(2):738–52. https://doi.org/10.4143/crt.2015.102.

129. Shin YS, Cha HY, Lee BS, et al. Anti-cancer effect of luminacin, a marine microbial extract, in head and neck squamous cell carcinoma progression via autophagic cell death. Cancer Res Treat. 2016;48(2):738–52. https://doi.org/10.4143/crt.2015.102.

130. Jung DW, Kim J, Che ZM, et al. A triazine compound S06 inhibits proinvasive crosstalk between carcinoma cells and stromal fibroblasts via binding to heat shock protein 90. Chem Biol. 2011;18(12):1581–90. https://doi.org/10.1016/j.chemb iol.2011.10.001.

131. Cichoń MA, Szentpetery Z, Caley MP, Papadakis ES, Mackenzie IC, Brennan CH, O'Toole EA. The receptor tyrosine kinase Axl regulates cell-cell adhesion and stemness in cutaneous squamous cell carcinoma. Oncogene. 2014;33(32):4185–92. https://doi.org/10.1038/onc.2013.388.

132. Martins VL, Caley MP, Moore K, et al. Suppression of TGFβ and angiogenesis by type VII collagen in cutaneous SCC. J Natl Cancer Inst. 2015;108(1):djv293. https://doi.org/10.1093/jnci/djv293.

133. Neuffer SJ, Cooper CD. Zebrafish syndromic albinism models as tools for understanding and treating pigment cell disease in humans. Cancers. 2022;14(7):1752. https://doi.org/10.3390/cancers14071752.
138. Wen-Rui L, Cheng-Rang L, Lin L. Zebrafish model of hereditary pigmentary disorders. Int J Dermatol Venereol. 2019;2(4):216–20. https://doi.org/10.1007/s12885-017-3647-0.

139. Runtuwene V, van Eekelen M, Overvoorde J, et al. Noonan syndrome gain-of-function mutations in NRAS cause zebrafish gastrulation defects. Dis Model Mech. 2011;4(3):393–9. https://doi.org/10.1242/dmm.007112.

140. Thomas AC, Zeng Z, Rivière JB, et al. Mosaic activating mutations in GNA11 and GNAQ Are associated with phakomatosis pigmentovascularis and extensive dermal melanocytosis. J Invest Dermatol. 2016;136(4):770–8. https://doi.org/10.1016/j.jid.2015.11.027.

141. Jindal GA, Goyal Y, Yamaya K, et al. In vivo severity ranking of Ras pathway mutations associated with developmental disorders. Proc Natl Acad Sci USA. 2017;114(3):510–5. https://doi.org/10.1073/pnas.1615651114.

142. Richardson R, Slanchev K, Kraus C, Knyphausen P, Eming S, Hammerschmidt M. Adult zebrafish as a model system for cutaneous wound-healing research. J Invest Dermatol. 2013;133(6):1655–65. https://doi.org/10.1038/jid.2013.16.

143. Naomi R, Bahari H, Yazid MD, Embong H, Othman F. Zebrafish as a model system to study the mechanism of cutaneous wound healing and drug discovery: advantages and challenges. Pharmaceuticals. 2021;14(10):1058. https://doi.org/10.3390/ph14101058.

144. Sonawane M, Carpio Y, Geisler R, Schwarz H, Maischein HM, Nuesslein-Volhard C. Zebrafish penner/lethal giant larvae 2 functions in hemidesmosome formation, maintenance of cellular morphology and growth regulation in the developing basal epidermis. Development. 2005;132(14):3255–65. https://doi.org/10.1242/dev.01904.

145. Carney TJ, von der Hardt S, Sonntag C, Amsterdam A, Topczewski J, Hopkins N, Hammerschmidt M. Inactivation of serine protease Matriptase1a by its inhibitor Hai1 is required for epithelial integrity of the zebrafish epidermis. Development. 2007;134(19):3461–71. https://doi.org/10.1242/dev.004556.

146. Mathias JR, Dodd ME, Walters KB, Rhodes J, Kanki JP, Look AT, Hutenlocher A. Live imaging of chronic inflammation caused by mutation of zebrafish Hai1. J Cell Sci. 2007;120(Pt 19):3372–83. https://doi.org/10.1242/jcs.009159.

147. Webb AE, Driever W, Kimelman D. psoriasis regulates epidermal development in zebrafish. Dev Dyn. 2008;237(4):1153–64. https://doi.org/10.1002/dvdy.21509.

148. Candel S, de Oliveira S, López-Muñoz A, et al. Tnfa signaling through tnfr2 protects skin against oxidative stress-induced inflammation. PLoS Biol. 2014;12(5):e1001853. https://doi.org/10.1371/journal.pbio.1001853.

149. Galindo-Villegas J. Recent findings on vertebrate developmental immunity using the zebrafish model. Mol Immunol. 2016;69:106–12. https://doi.org/10.1016/j.molimm.2015.10.011.

150. Galindo-Villegas J, García-Moreno D, de Oliveira S, Meseguer J, Mulero V. Regulation of immunity and disease resistance by commensal microbes and chromatin modifications during zebrafish development. Proc Natl Acad Sci USA. 2012;109(39):E2605-14. https://doi.org/10.1073/pnas.1209920109.

151. Galindo-Villegas J, Montalban-Arques A, Liarte S, et al. TRPV4-mediated detection of hypoxic stress by skin keratinocytes activates developmental immunity. J Immunol. 2016;196(2):738–49. https://doi.org/10.4049/jimmunol.1501729 (published correction appears in J Immunol. 2016 Apr 15;196(8):3494).

152. Martínez-Navarro FJ, Martínez-Menchón T, Mulero V, Galindo-Villegas J. Models of human psoriasis: zebrafish the newly appointed player. Discover Oncology (2022) 13:48 | https://doi.org/10.1007/s12672-022-00511-3.

153. Yu CI, Chen CY, Liu W, et al. Sandensolide induces oxidative stress-mediated apoptosis in oral cancer cells and in zebrafish xenografts. PLoS One. 2014;9(10):e110667. https://doi.org/10.1371/journal.pone.0110667.

154. Postel R, Margadant C, Fischer B, et al. Kindlin-1 mutant zebrafish as an in vivo model system to study cell adhesion mechanisms in the epidermis. J Invest Dermatol. 2013;133(9):2180–90. https://doi.org/10.1038/jid.2013.154.

155. Topczewska JM, Postovit LM, Margaryan NV, et al. Embryonic and tumorigenic pathways converge via Nodal signaling: role in melanoma aggressiveness. Nat Med. 2006;12(8):925–32. https://doi.org/10.1038/nm16100387.

156. Ceol CJ, Houvras Y, Jane-Valbuena J, et al. The histone methyltransferase SETDB1 is recurrently amplified in melanoma and accelerates tumour cell migration. J Invest Dermatol. 2016;136(4):770–8. https://doi.org/10.1016/j.jid.2015.11.027.

157. Tang Q, Abdelfattah NS, Blackburn JS, et al. Optimized cell transplantation using adult rag2 mutant zebrafish. Nat Methods. 2014;11(18):821–4. https://doi.org/10.1038/nmeth.3031.

158. Tang Q, Moore JC, Ignatius MS, et al. Single-cell imaging of normal and malignant cell engraftment into optically clear prkdc-null SCID zebrafish. J Exp Med. 2016;213(23):2755–89. https://doi.org/10.1084/jem.20160378.

159. Moore JC, Tang Q, Yordán NT, et al. Imaging tumour cell heterogeneity following cell transplantation into optically clear prkdc-null SCID zebrafish embryo. Dev Cell. 2019;48(4):554-72.e7. https://doi.org/10.1016/j.devcel.2019.01.014.

160. Benjamin DC, Hynes RO. Intravital imaging of metastasis in adult zebrafish. BMC Cancer. 2017;17(1):660. https://doi.org/10.1186/s12885-017-3647-0.

161. Hyenne V, Ghoroghi S, Collot M, et al. Studying the fate of tumor extracellular vesicles at high spatiotemporal resolution using the zebrafish embryo. Dev Cell. 2019;48(4):554-72.e7. https://doi.org/10.1016/j.devcel.2019.01.014.

162. Lee LM, Seftor EA, Bonde G, Cornell RA, Hendrix MJ. The fate of human malignant melanoma cells transplanted into zebrafish embryos: assessment of migration and cell division in the absence of tumor formation. Dev Dyn. 2005;233(4):1560–70. https://doi.org/10.1002/dvdy.20471.

163. Halldí M, Ton C, Seng WL, McGrath P. Human melanoma cells transplanted into zebrafish proliferate, migrate, produce melanin, form masses and stimulate angiogenesis in zebrafish. Angiogenesis. 2006;9(3):139–51. https://doi.org/10.1007/s10456-006-9040-2.

164. Nicoli S, Presta M. The zebrafish/tumor xenograft angiogenesis assay. Nat Protoc. 2007;2(11):2918–23. https://doi.org/10.1038/nprot.2007.412.

165. Nicoli S, Ribatti D, Cotelli F, Presta M. Mammalian tumor xenografts induce neovascularization in zebrafish embryos. Cancer Res. 2007;67(7):2927–31. https://doi.org/10.1158/0008-5472.CAN-06-4268.

166. Yan C, Brunson DC, Tang Q, et al. Visualizing engrafted human cancer and therapy responses in immunodeficient zebrafish. Cell. 2019;177(7):1903-14.e14. https://doi.org/10.1016/j.cell.2019.04.004.
Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.