Antiangiogenic molecules from marine actinomycetes and the importance of using zebrafish model in cancer research

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ABSTRACT

Blood vessel sprouting from pre-existing vessels or angiogenesis plays a significant role in tumour progression. Development of novel biomolecules from marine natural sources has a promising role in drug discovery specifically in the area of antiangiogenic chemotherapeutics. Symbiotic actinomycetes from marine origin proved to be potent and valuable sources of antiangiogenic compounds. Zebrafish represent a well-established model for small molecular screening and employed to study tumour angiogenesis over the last decade. Use of zebrafish has increased in the laboratory due to its various advantages like rapid embryo development, optically transparent embryos, large clutch size of embryos and most importantly high genetic conservation comparable to humans. Zebrafish also shares similar physiopathology of tumour angiogenesis with humans and with these advantages, zebrafish has become a popular model in the past decade to study on angiogenesis related disorders like diabetic retinopathy and cancer. This review focuses on the importance of antiangiogenic compounds from marine actinomycetes and utility of zebrafish in cancer angiogenesis research.

1. Introduction

Blood vessels’ sprouting from preexisting vasculature is angiogenesis, which can occur at both physiological as well as pathological conditions like wound healing, placentation, embryogenesis, inflammatory disorders and tumour growth [1, 2]. In tumour angiogenesis, tumour cell releases certain molecules that signal the host tissue and activate specific genes to make protein that boost development of novel blood vessels (Figure 1) [3]. Vascular endothelial growth factor (VEGF) is the key angiogenic determinant factor of angiogenesis (VEGF) and targeting its expression; thereby blocking the VEGF signaling cascade would be significantly useful in the development of new anticancer drugs [4]. Many novel bioactive molecules from natural sources are undergoing clinical trials to downregulate vegf and thereby disrupt the growth of angiogenic vessels [5, 6]. Natural products from marine sources are increasing popularity in drug discovery, especially the marine actinomycetes plays major role in development of novel bioactive compounds.

The idea of employing marine bioactive molecules to target angiogenic growth factors has been of a great importance in the past three decades after the substantial contribution by Dr Judah Folkman [7]. Marine invertebrates such as molluscs, gorgonia, soft coral, sponges, sponge-associated bacteria and actinomycetes, have been widely explored for possible angiogenic inhibitors [8]. Small molecular compounds from marine origin have become important in cancer research as well as in the study of antibacterial, antifungal, antiviral and anti-coagulant properties [9, 10]. It is evident that conventional treatment for cancer has many side effects and it is crucial to develop natural products based anticancer therapies in future. In angiogenic drug discovery efforts, rodent models have dominated to date, however, these models are not suitable for large-scale drug screening when compared to the advantages of zebrafish which requires minimal labor, resources and time. Furthermore, ethical issues in the usage of rodents have made their usage even more limited [11, 12, 13]. Zebrafish is an extensive model organism to study small molecular drug interactions as it provides a...
series of advantages like optical transparency, rapid development, and high number of offspring. Marine actinomycetes are distributed widely and thus, discovering novel antiangiogenic compounds from them can serve as promising candidates for cancer drug discovery. Figure 2 depicts the distribution of actinomycetes from marine sources, antiangiogenic small molecules discovered from marine actinomycetes and the importance of utilizing zebrafish in cancer research [14]. Thus, this review focuses on the utilization of zebrafish as a relevant model organism in antiangiogenic drug discovery mainly about marine symbiotic actinomycetes and drug screens.

2. Antiangiogenic agents from marine actinomycetes

Marine sources are rich in secondary metabolites and there are many compounds reported to possess anticancer properties. In a recent review, it has been elucidated that more than 45 compounds from marine origin are shown to have antiangiogenic potential and 10 of them have already entered clinical trials at different phases for cancer therapy [8]. These compounds include terpenes, saccharides, saponins, macrocycles, xanthones, peptides, alkaloids and pyrones which display a great structural and chemical diversity and also these compounds downregulate angiogenesis by altering distinct targets due to their unique structures. These angiogenesis inhibitors act directly on the endothelial cells or other growth factors of the angiogenic cascade (Figure 3) and they hinder the growth of the endothelial cells by arresting the cell cycle during mitosis or by causing DNA damage leading to apoptosis [15].

Actinomycetes are filamentous Gram-positive bacteria which belongs to the phylum Actinobateria, are considered to be the largest group in the bacterial domain [16]. Bioactive molecules from actinomycetes are reported to be the highest among the other bacterial species which is almost 45 percent of the overall metabolites reported [17]. Streptomyces is the major group among actinomycetes which has produced around 7,600 compounds [17] and they have also produced clinically important antitumour agents [18, 19]. The undesirable side effects and high toxicity of already available chemotherapy drugs for cancer treatment makes the researches to discover novel antitumour drugs from marine origin or phytochemicals which have no/less side effects when compared to conventional therapy [20]. Marine actinomycetes are unique in producing secondary metabolites when compared to other microorganisms from terrestrial origin; with many

![Figure 1. The process of angiogenesis.](image1.png)

![Figure 2. Importance of zebrafish model to study antiangiogenic compounds from marine actinomycetes (a. Distribution of actinomycetes from marine sources [14], b. Antiangiogenic biomolecules from marine actinomycetes, c. Advantages of zebrafish model in cancer research).](image2.png)
pharmacologically important activities like anti-oxidant, anti-inflammation and antitumour properties [21, 22, 23, 24, 25]. Table 1 lists some of the important antitumour compounds from marine actinomycetes. Marine microorganisms possess unique features and thus, they might synthesize different secondary metabolites in their challenging habitats [100]. Most important derivatives from marine actinomycetes which possess antiangiogenic potential are described in Table 2 and their structures are shown in Figure 4.

3. Zebrafish - a suitable model for angiogenesis research

Zebrafish model is widely used in angiogenesis study as the circulation starts after 24 hours post-fertilization (hpf), and the vascular system bears a strong similarity to that of humans. In the early embryonic development, blood vessels and organ formation can be easily visualized in the transparent embryos and larvae of both wildtype and transgenic species making it a viable model for angiogenesis research [105]. Therefore, this advantage of zebrafish plays an important role in studying tumour angiogenesis, which is crucial for cancer progression and metastasis and also serves as targets for antitumour therapeutics. Staining of vascular endothelial cells of zebrafish by a fluorescent protein can render the observation of newly formed blood vessels in the earliest tumour progressive stage. Zebrafish also serves as a tumour metastasis model; due to its transparent embryos and larvae the metastasizing tumour cells can be exactly traced by the fluorescent-stained tumour cells at the cellular level [106]. Furthermore, the large clutch size of embryos and inexpensiveness of zebrafish make them easily amenable for the large-scale drug screen in antiangiogenic drug discovery and efficacy.

3.1. Zebrafish transgenic models in tumour angiogenesis

Transgenic technology has improved the characteristic in vivo imaging capabilities of zebrafish larvae and embryos. A dissecting microscope is sufficient to visualize the blood flow and vessel development in early embryos and larvae, yet tissue specific expression of fluorescent proteins is required to study the vasculature in detail (Figure 5) [107]. Phenotypic changes and cell shape abnormalities with live specimens can be studied in detail by confocal microscopy and time-lapse imaging techniques and thus, formation of vasculature has been explained with the use of molecular markers in detail, both from the cellular and anatomical point of view [108, 109, 127]. Based on gene-specific promoters, transgenic zebrafish mutant lines were developed with vascular-specific phenotypes and both heterologous and autologous promoters have been shown to work. Zebrafish transgenic mutant lines which have been developed to study the vasculature is given in Table 3. The promoter closely similar to mammalian species was used previously; before the availability of whole genome sequence of zebrafish [128].

Molecular traces have been employed to study the formation of vasculature in zebrafish, during the embryonic development and thus the vascular anatomy has been well documented which has proven to share quiet a high percentage of resemblance with higher order vertebrates [108, 109, 130]. Based on gene-specific promoters, transgenic zebrafish mutant lines were developed with vascular-specific phenotypes and both heterologous and autologous promoters have been shown to work. Zebrafish transgenic mutant lines which have been developed to study the vasculature is given in Table 3. The promoter closely similar to mammalian species was used previously; before the availability of whole genome sequence of zebrafish [128].

Figure 3. Marine derived drugs targeting tumour angiogenesis.
Table 1. Antitumour compounds produced by marine actinomycetes.

| Structural type                                      | Compound                                                                 | Organism                                                                 | Reference                                                                 |
|------------------------------------------------------|--------------------------------------------------------------------------|--------------------------------------------------------------------------|--------------------------------------------------------------------------|
| Indole                                               | 3,6-disubstituted indoles                                                | *Streptomyces* sp. BL-49-58-005                                         | [26]                                                                     |
| Indole                                               | Streptoclin                                                              | *Streptomyces* sp. 04DH1 10                                              | [27-29, 30]                                                              |
| Polyketide                                           | 1-hydroxy-1-norresistomycin                                              | *Streptomyces* chinensis AUBN1/7                                         | [31-32]                                                                  |
| Polyketide                                           | 1,8-dihydroxy-2-ethyl-3-Methanithraquinone                               | *Streptomyces* sp. FX-58                                                 | [33]                                                                     |
| Polyketide                                           | Actinofuranones                                                         | *Streptomyces* sp. CNQ766                                                | [34]                                                                     |
| Polyketide                                           | Arenilolides                                                            | *Salinispora arenicola* CNR-005                                          | [35]                                                                     |
| Polyketide                                           | Aureovericillactam                                                      | *Streptomyces aureovericillatus* NPS001583                               | [36]                                                                     |
| Polyketide                                           | Chalcomycin                                                             | *Streptomyces* sp. M491                                                 | [37]                                                                     |
| Polyketide                                           | Chalcomycin B                                                           | *Streptomyces* sp. B4842                                                | [38]                                                                     |
| Polyketide                                           | Chartreusin                                                             | *Streptomyces* sp. QD518                                                | [39]                                                                     |
| Polyketide                                           | Cyanosporasides                                                         | *Salinispora pacifica* CNS103                                            | [40]                                                                     |
| Polyketide                                           | Daryamides                                                              | *Streptomyces* sp. CNQ-085                                               | [41]                                                                     |
| Polyketide                                           | Fridamycin D                                                            | *Streptomyces* sp. B6921                                                | [42]                                                                     |
| Polyketide                                           | Griseorhodin A                                                          | *Streptomyces* sp. JP9                                                  | [43, 44]                                                                 |
| Polyketide                                           | Himalomycins                                                            | *Streptomyces* sp. B6921                                                | [42]                                                                     |
| Polyketide                                           | IB-0028                                                                 | *Actinomadura* sp. BL-41-PO13-046                                       | [45, 46]                                                                 |
| Polyketide                                           | IB-96212                                                                | *Micromonospora* sp. L-25-ES25-008                                       | [47, 48]                                                                 |
| Polyketide                                           | Komodoquinones                                                          | *Streptomyces* sp. KS3                                                  | [49, 50]                                                                 |
| Polyketide                                           | Manumycin C                                                             | *Streptomyces* sp. M405                                                | [51]                                                                     |
| Polyketide                                           | Marinomycins                                                            | *Marinispora* sp. CNQ-140                                               | [52]                                                                     |
| Polyketide                                           | Marmycins                                                               | *Streptomyces* sp. CNH090                                               | [53]                                                                     |
| Polyketide                                           | Nonactin                                                                | *Streptomyces* sp. KORDI-3238                                           | [54]                                                                     |
| Polyketide                                           | Pacificanones                                                           | *Salinispora pacifica* CNS-237                                          | [55]                                                                     |
| Polyketide                                           | Parimycin                                                               | *Streptomyces* sp. B8652                                                | [56]                                                                     |
| Polyketide                                           | Piericidins                                                             | *Streptomyces* sp. YMI-14-060                                          | [57, 58]                                                                 |
| Polyketide                                           | Rabelomycins                                                            | *Streptomyces* sp. B6921                                                | [42]                                                                     |
| Polyketide                                           | Restitoflavine                                                          | *Streptomyces* chinensis AUBN1/7                                         | [31-32, 59]                                                              |
| Polyketide                                           | Resistomycin                                                            | *Streptomyces* sp. B8005                                                | [32]                                                                     |
| Polyketide                                           | Salini ketals                                                           | *Salinispora arenicola* CNR-005                                          | [60]                                                                     |
| Polyketide                                           | Salinipyrones                                                           | *Salinispora pacifica* CNS-237                                          | [55]                                                                     |
| Polyketide                                           | Sporolides                                                              | *Salinispora tropica* CNB-392                                           | [61]                                                                     |
| Polyketide                                           | SS-228 Y                                                                | *Chainsia* sp. SS-228                                                   | [62, 63]                                                                 |
| Polyketide                                           | Tetraclomycin D                                                         | *Streptomyces* sp. B8005                                                | [32]                                                                     |
| Polyketide                                           | Triocarcenin                                                             | *Streptomyces* sp. isolate B8652                                       | [64]                                                                     |
| Non-ribosomal peptide                                | Arenamides                                                              | *Salinispora arenicola* CN-088                                           | [65]                                                                     |
| Non-ribosomal peptide                                | Lucentamycins                                                           | *Nocardiosis lucentensis* CNR-712                                       | [66]                                                                     |
| Polyketide/non-ribosomal peptide                     | Lajollamycin                                                            | *Streptomyces nodus* NPS007994                                         | [67]                                                                     |
| Non-ribosomal peptide                                | Mecherecharmycins                                                      | *Thermoactinomycetes* sp. YM3-251                                       | [68]                                                                     |
| Non-ribosomal peptide                                | Piperazimycins                                                          | *Streptomyces* sp. CNQ-593                                              | [69]                                                                     |
| Non-ribosomal peptide                                | Proximicincs                                                           | *Verrucosporis* sp. MG-7                                               | [70, 71, 72]                                                             |
| Polyketide/non-ribosomal peptide                    | Salinosporamides                                                        | *Salinispora tropica* CNB-392                                           | [61, 73, 74, 75, 76]                                                    |
| Non-ribosomal peptide                                | Thiocoraline                                                            | *Micromonospora* sp. L-13-ACM2-092                                      | [77, 78]                                                                 |
| Isoprenoid                                           | 4α,8α-dimethyl-6-[(2-methylpropenyl氧基)-3,4,α,4β,5,6,8α,9-octahydro-1H-phenanthren-2-one | *Actinobacteria* sp. MS1/7                                               | [79]                                                                     |
| Isoprenoid                                           | Altemicidin                                                             | *Streptomyces* sioguensis SA-1758                                       | [80, 81]                                                                 |
| Isoprenoid                                           | Chlorinated dihydroquinones                                            | *Actinomycete* isolate CNQ-525                                          | [82]                                                                     |
| Isoprenoid                                           | Marinones                                                               | *Actinomycete* isolate CNH-099                                          | [83, 84, 85]                                                             |
| Isoprenoid                                           | T-Muurolol                                                              | *Streptomyces* sp. M491                                                | [37, 86]                                                                 |
| Indolocarbazole                                       | Arcyriaflavin A                                                         | *Actinomycete* sp. Z2039-2                                              | [87]                                                                     |
| Indolocarbazole                                       | K252c                                                                   | *Actinomycete* strain Z2039-2                                           | [87]                                                                     |
| Indolocarbazole                                       | Staurosporins                                                           | *Streptomyces* sp. KS3                                                 | [39, 50, 88]                                                             |

(continued on next page)
cell markers are employed to increase the possibility to visualize the migratory and proliferative behaviors of single cells, and various other cell types during the embryo-to-larva transition. Two different cell types were observed simultaneously by combining transgenic lines expressing different fluorescent proteins [113, 133, 134, 135]. Additionally, by using the combination of cell and nuclear membrane specific fluorescent tags, researchers have reported to study the single cell morphological dynamics in living larvae during vascular development [136]. Zebrafish transgenic lines development has been of a much greater utility in studying induced gene expression and also tissue specific gene expression [137]. Thus, these strategies facilitated the study of the sequence of events taking place during the formation of early circulatory loop in zebrafish embryos. The intersegmental vessels are the important angiogenic vessels, whose development is of a greater importance because of its characteristics and high accessibility feature in the zebrafish embryos. The intersegmental vessels are the important angiogenic vessels, whose development is of a greater importance because of its characteristics and high accessibility feature in the zebrafish embryos and larvae; these vessels emanate from dorsal aorta into the embryonic tail and trunk region, and finally grow into the anastomosing dorsal longitudinal vessels [138]. Experimental analysis of blood vessel development in zebrafish embryogenesis was carried out using two common methods namely immunohistochemistry and in situ hybridization for the visualization of protein and gene expression. But these methods were not specifically developed to study zebrafish vasculature, but various other protocols and tools are currently available that enable these strategies [139, 140]. Regardless of its popularity and success, the researchers using zebrafish model must also contemplate their work by extending their research on other higher vertebrates or mammalian systems, for further clinical applications in future.

3.2. Zebrafish in drug screens

The rationale of zebrafish usage for high-throughput drug screening of marine bioactive compounds as become popular in the past decade as these animals involve only sub-milligram quantities for hit selection and validation and are easily pliable to multi-well plates for the reason that they have small sized embryos and larvae [11, 141, 142]. The quantity of marine bioactive compounds for primary screening purposes is limited and it is yet another disadvantage of rodents in marine drug discovery as they require higher quantity for drug screening. As discussed earlier, optical transparency of zebrafish embryos until 5 days post-fertilization (dpf) aids easy visualization of tissues and organs and this feature, allows researchers to employ zebrafish transgenic lines coupled with fluorescently labeled organs and cells, and to study the vascular patterning by developing assay methods for chemical and genetic screening approaches [143, 144]. Significantly, several small bioactive compounds identified in zebrafish possess anticancer properties and are currently in clinical trial phase [141]. Zebrafish can also be used for phenotype-based drug discovery which allows the identification of small molecules independently of their mode of action [141, 142]. Zebrafish embryos and larvae have been used in drug screening strategies so far and anti-angiogenic properties of marine compounds studied in zebrafish model are discussed in Table 4.

3.3. Zebrafish Xenograft model

Xenografting is a pre-clinical tool used by researchers in the recent times to evaluate drug responses and to study tumour metastasis [151].

Table 2. Important derivatives from marine actinomycetes which possess antiangiogenic potential.

| Compound | Marine organism Source | Action | Reference |
|----------|------------------------|--------|-----------|
| Streptopyrrolidine | Streptomyces sp | Inhibition of tube formation in HUVECs | [98] |
| Cyclo-(L-Pro-L-Met) | Nocardisporangium sp. 03N67 | Antiangiogenesis activity against human umbilical vein endothelial cells (HUVECs) | [101] |
| Streptochlorin | Streptomyces strain 04D110 | • Inhibition of in vitro growth of human leukemia K-562 cells with an IC50 of 1.05 μg/mL significantly • Potent antiangiogenic agent by inducing ROS-mediated apoptosis and inhibits TNF-α-induced NF-κB activation. • Antiangiogenic potential by downregulating the expression of VEGF. | [27, 28, 29, 30] |
| Lynamicins | Marinicipora sp. NPS12745 | • Potent antitumour and antiangiogenic properties • Reduction of resistance mediated by transporter ABCG2 | [102, 103] |
| Marinomobil | Solmisporangium tropica | Potential anticancer agent and is currently undergoing Phase-I clinical trial. | [8, 104] |
| Thiocoraline | Micromonospora sp. L-13-ACM2-092 | • Potent antitumour activity against melanoma MEL288, human lung adenocarcinoma A549, and marine leukemia P388 | [77] |
Figure 4. Structures of marine actinomycetes derived compounds that possess antiangiogenic potential.
Zebrafish is established as an efficient model for human tumour xenotransplantation (XT), specifically human leukemias and lymphomas. Absence of adaptive immune system in zebrafish larvae until 28hpf makes them a suitable XT model, with no constraint for immunosuppression. Likewise, the zebrafish XT system allows real time observation and imaging of tumour-cell crescendos in a live animal microenvironment. High conservation is observed in the developmental process of hematopoiesis of zebrafish, making it a robust model to study normal and abnormal blood vessel development and disorders especially in blood cancer research. Therefore, zebrafish can be utilized as a pre-clinical screening model to establish patient-derived cancer cell xenotransplantation and develop novel possibilities for personalized medicine. Table 5 gives important xenograft transplantation cancer models in zebrafish. The first studied xenotransplantation of human cells into zebrafish [153] led many researchers to use the zebrafish embryos to establish the factors underlying in the other sides of cancer biology which includes cancer-induced angiogenesis, cancer cell invasion and metastasis [176, 177]; cancer cells interaction with host cell [178]; and screening of drugs [179, 180]. In a recent study using zebrafish xenograft model (CDX), antiangiogenic effectiveness of ramucirumab, apatinib, regorafenib and cabozantinib was evaluated for the intersegmental vessels (ISVs) and subintestinal veins (SIVs) formation, in which all the four drugs exhibited antiangiogenic potential in the Tg (fl1-1:EGFP) zebrafish embryos [181]. Significantly, the laboratory observation

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**Table 3. Transgenic zebrafish lines developed to study and visualize the vasculature.**

| Line                        | Expression | Gene                     | Reference |
|-----------------------------|------------|--------------------------|-----------|
| Tg(dbh:EGFP)                | Endothelial cells | Notch ligand | [110]    |
| Tg(Tie2:EGFP)               | Endothelial cells | Tie-2 receptor tyrosine kinase | [111]    |
| TgBAC(dbh:GAL4FF)           | Endothelial cells | Notch ligand | [112]    |
| Tg(--):GFPy1                | Endothelial cells, cytoplasmic | Transcription factor Fli-1 | [113]    |
| Tg(fl1-meqGFP)y7            | Endothelial cells, nuclear | Transcription factor Fli-1 | [114]    |
| Tg(5xUAS:cdh5-EGFP)         | Pan-endothelial | VE-cadherin | [115]    |
| Tg(--):YFP                  | Pan-endothelial | VEGF | [116]    |
| TgBAC(cdh5:Citrine)         | Pan- endothelial | VE-cadherin | [117]    |
| TgBAC(cdh5:GAL4FF)          | Pan- endothelial | VE-cadherin | [118]    |
| TgBAC(--):Citrine           | Pan-endothelial | VEGF | [119]    |
| Tg(kdr:eGFP)+843            | Angioblast/endothelial precursors | VEGF2/Flk1/kdr/Vegfr4 | [120]    |
| Tg(kdr-G:RCFP)              | Angioblast/endothelial precursors | VEGF2/Flk1/kdr | [121]    |
| Tg(gata1:dsRed):ud2         | Blood cells | Transcription factor GATA-1 | [122]    |
| Tg(gata2::GFP)              | Blood cells | Transcription factor GATA-2 | [122]    |
| Tg(hap70::canotch3-EGFP)    | Perivascular | Notch3 intracellular domain | [123]    |
| Tg(gata1::GFP)              | Erythroid lineage | Transcription factor GATA-1 | [124]    |
| Tg(fl1-7.8fa-c::GFP):nc3    | Endocardial and myocardial cells | Transcription factor GATA-4 | [125]    |
| Tg(myt17::GFP)              | Myocardial cells | Cardiac myosin light chain 2 | [126]    |

Adapted from Baldessari and Mione (2008), Kamei et al. (2010) and Schuermann et al. (2014).

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**Table 4. List of marine compounds with antiangiogenic properties studied using zebrafish model.**

| Compound                  | Action                      | Targeted molecules                  | Reference |
|---------------------------|-----------------------------|-------------------------------------|-----------|
| Solomomonamide A          | Antiangiogenesis            | ERK1/2 and Akt phosphorylation      | [145]    |
| Catunaregin               | Antiangiogenesis            | Modulating phosphorylation of Akt and eNOS | [146]    |
| Somocystinamide A         | Antiangiogenesis by inhibiting tube formation of endothelial cells | Caspase-8-expressing tumours | [147]    |
| Stellettin B              | Decreased blood vessel formation in developmental zebrafish | VEGF transcriptional expression | [148]    |
| Crambesicidin 816         | Antitumour effect           | Caspase-3 cleavage and activation. | [149]    |
| Bromophenol BBDE          | Antiangiogenesis inhibiting sub-intestinal vessel formation | VEGF/VEGFR | [150]    |
Table 5. List of Human Cancer xenograft transplantation models in zebrafish.

| Tumours                              | Transplant stage | Site of injection | Observation                                                                 | Reference |
|--------------------------------------|------------------|------------------|----------------------------------------------------------------------------|-----------|
| Melanoma and colorectal cancer       | 48 hpf           | Yolk sac, hind brain ventricle | Inhibition of vascularization by VEGFR2 inhibitor - SU5416.                 | [152]     |
| (both murine)                        |                  |                  |                                                                             |           |
| Melanoma, Uveal melanoma             | Blastula         | Blastodisc       | • Studied tumor cell plasticity and investigated tumor microenvironment interactions. | [153]     |
| 48hpf                                | Yolk sac         |                  | • Large scale drug screening and drug discovery                             | [154]     |
| Prostate Cancer (androgen dependent and independent) | 48 hpf | Yolk sac | • Silencing of tyrosine kinase SYK prevented cancer cell dissemination. | [155]     |
|                                      |                  |                  | • Xenograft using LNCaP in zebrafish treated with exogenous testosterone - increased cancer cell proliferation | [156]     |
| Colorectal cancer                    | 48 hpf           | Yolk sac         | • Activation of by intrinsic apoptotic signaling by Marine guanidine alkaloids in tumour regression. | [149]     |
|                                      |                  |                  | • Efficacy of Bromelanin in tumour regression.                              | [157]     |
| Pancreatic cancer                    | 48 hpf           | Yolk sac         | Evaluation of tumour cell invasion and micrometastasis with transgenic zebrafish | [158]     |
| Breast cancer                        | 48 hpf           | Yolk sac, Duct of Cuvier | • Patient-derived material (PDX) model in bone metastasis research. | [159]     |
|                                      |                  |                  | • Role of SOX2 interaction with AKT signalling in breast cancer.           | [160]     |
| Breast cancer, non-invasive and metastatic | 48 hpf | Duct of Cuvier | TGF-β receptor kinase inhibitors for blocking and inhibiting TGF-β signaling. | [161]     |
| Retinoblastoma                       | 48 hpf           | Yolk sac; brain  | Orthoptic zebrafish model to understand the invasive and metastatic nature of retinoblastoma | [162]     |
| Glioblastoma                         | 52 hpf           | Yolk sac; brain  | • Changes in the cell heterogeneity after treatment with chemotherapy on tumour. | [163]     |
|                                      |                  |                  | • Model for detection of BBB (Blood-Brain Barrier) penetration of TNB.    | [164]     |
|                                      |                  |                  | • RECQ1 helicase, a promising molecular target in the glioblastomotherapy and high throughput screening | [165]     |
| Gastrointestinal tumours pancreas, stomach, colon | 48 hpf | Yolk sac; liver | Inhibition of growth and metastasis in xenografted cells by targeting EGFR and its downstream signalling molecules AKT/ERK by Triphala | [166]     |
| Oral squamous cell carcinoma         | 48 hpf           | Yolk sac         | Induction of apoptosis by Sandenolide in Oral cancer.                     | [167]     |
| Non-small-cell lung cancer (NSCLC)   | 48 hpf           | Yolk sac         | • Bevacizumab, endostar and apatinib effects and its toxicity were analyzed. | [168]     |
| Ewing sarcoma (EWS)                  | 25 dpf           | Yolk sac, Eye vessels | Nutlin-3, a tp53 activator, and YK-4-279, a EWSR1-ETS inhibitor as a Combinational therapy was studied. | [169]     |
| 48 hpf                               | Yolk sac         |                  | Drug efficacy and sensitivity was analysed using zebrafish PDX. Progression of cancer by cell dissemination and homing to bone marrow were investigated. | [170]     |
| MM, Waldenstrom's macroglobulinemia  | 48hpf            | Yolk sac; Pericardium | Drug efficacy and sensitivity was analysed using zebrafish PDX. Progression of cancer by cell dissemination and homing to bone marrow were investigated. | [170]     |
| AML                                  | 48 hpf           | PC vein          | Inhibitory effect of imatinib and other antileukemic drugs.                | [171]     |
| Glioblastoma, melanoma, breast cancer, RMS | Adult | Peri-ocular muscle | A double mutant immunodeficient zebrafish to study cancer xenotransplantation. | [172]     |
| MM cells from plasma MM cells from bone marrow | 48 hpf | Yolk sac, Pericardium | Drug sensitivity or resistance were investigated using zebrafish model. | [173]     |
| AML, HCC                             | 48 hpf           | Yolk sac, Trunk near dorsal aorta; heart | Treatment with busulfan successfully enabled xenograft AML cells and HCC cells into adult zebrafish | [174]     |
| CML, HCC, prostate cancer            | 48 hpf           | Yolk sac, Trunk near dorsal aorta | Model for xenotransplantation and drug screening by introducing cancer stem-like cells. | [175]     |

of developing a zebrafish tumour model and its response to chemotherapeutics is comparable to mouse xenograft models [182]. With these features, zebrafish can also be considered as a vital XT model to study and identify marine bioactive molecules.

4. Summary and conclusion

The established angiogenic inhibitors or small bioactive compounds from marine symbiotic actinomycetes provide hope for reducing the morbidity and mortality from metastatic cancers and other carcinomas. Though, it is reported to have successful results with the use of established antiangiogenic drugs which have entered clinical trials, long term survival benefits in cancer patients can be achieved by combination therapy by combining small molecules with chemotherapy or radiation therapy. The neovascularization of cancer tissue as well as the growth of the tumour can be repressed by the use of angiogenesis-suppressors and thus might be helpful in the treatment of cancer and, in particular few bioactive compounds produced by genus Streptomyces, serves as a source of numerous antitumour drugs. As marine system consists of enormous beneficial microbes, it is important to take into account for drug discovery as there are innumerable compounds with novel structural diversity which are yet to be discovered from marine actinomycetes. Antiangiogenic marine bioactive compounds have been extensively found successful in cell lines study and rodent models, whereas their usage in zebrafish is still in emergence stage. Therefore, a most potential and successful animal model is required to study the novel drug efficacy in a cost-effective manner. As we discussed in detail above using zebrafish in marine drug discovery, they are already proven model in angiogenesis research, which helps us to identify and discover novel anticancer/antiangiogenic compounds from marine actinobacteria.
Declarations

Author contribution statement

All authors listed have significantly contributed to the development and writing of this article.

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Data availability statement

No data was used for the research described in the article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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