7

Modeling Tumor Angiogenesis with Zebrafish

Alvin C.H. Ma, Yuhan Guo, Alex B.L. He and Anskar Y.H. Leung
The University of Hong Kong,
Hong Kong

1. Introduction

Angiogenesis is process by which new blood vessels arise from endothelial cells in the existing vessels. In normal circumstances, the initiation, formation, maturation, remodeling and regression of endothelial cells in this process are strictly regulated. During tumor formation, the regulation of angiogenesis is disrupted and endothelial remodeling and regression are usually absent. Therefore, study on angiogenesis is of important relevance to cancer biology and therapeutic intervention (Carmeliet & Jain, 2000), especially in cancers where tumor growth depends on extensive vascularization (Folkman, 2002).

A number of in vitro and in vivo models have been used for the study of angiogenesis. These include an endothelial cell line derived from **h**uman **u**mbilical cord **v**ein **e**ndothelial **c**ells (HUVEC) (Jaffe et al., 1973) as well as a number of organ specific endothelial cell lines. With these cell lines, endothelial cell proliferation, differentiation and migration have been characterized. However, information about how endothelial cells interact with their neighboring cells is often lacking. In this regards, explant cultures (Brown et al., 1996; Jung et al., 2001) might be more representative of the complex interaction between endothelial and the supporting cells. Nevertheless, the issues of incomplete microenvironment, animal to animal variability and technical difficulties from relatively time-consuming and labor-intensive tissue isolation and culture might limit the application of these models.

In vivo models of angiogenesis have also been developed using chick embryo, rabbit and mouse (reviewed by Staton et al., 2009). They provide a more accurate physiological model of angiogenesis and when implanted with primary tumors or cancer cell lines, they can also provide important mechanistic insights to tumor angiogenesis. However, large-scale chemical screening with these models is difficult due to the cost and space needed for husbandry facilities.

Zebrafish has emerged as a model organism for the study of genetics and human diseases. Compare with other vertebrate models, this small tropical fish offers distinctive advantages. Firstly, zebrafish embryos are externally fertilized and optically transparent, allowing direct visualization during embryonic development. Secondly, these embryos are amenable to reverse genetic manipulation including gene knock-down, over-expression or transgenesis by microinjection. Thirdly, the high fecundity of zebrafish enables adequate experimental duplicates and facilitates high through-put forward genetic screening. Mating a single pair of adult zebrafish can produce hundreds of eggs in one day. Fourthly, stable tissue-specific transgenic fish-lines are available, allowing direct visualization of various developmental processes. Lastly, husbandry and maintenance of zebrafish colonies are space and cost effective.

Early zebrafish embryonic vascular development begins at around 12 hour-post-fertilization (hpf) when hemangioblasts first exist along the lateral plate mesoderm. Later at around 24 hpf, the development of dorsal aorta (DA) and dorsal vein (DV), forming the first circulation loop. Subsequently, angiogenesis including the development of inter-segmental vessels (ISV) and sub-intestinal veins (SIV) occurs. Important growth factors and associated receptor tyrosine kinases as well as Notch signaling pathway regulating mammalian vascular development are conserved in zebrafish (Liang et al., 1998; Habeck et al., 2002; Goishi and Klagsbrun, 2004; Siekmann and Lawson ND, 2007). Here, we explore the potential of using zebrafish in vivo to model and more importantly to screen potential therapeutic agents targeting tumor angiogenesis.

2. Zebrafish embryonic angiogenesis

During zebrafish embryonic development, angiogenesis is characterized by the sprouting of inter-segmental vessels in the trunk between each somite initiated around 24 hpf as well as the development of sub-intestinal veins initiated around 48 hpf (Isogai et al., 2001; Lawson and Weinstein, 2002a). Although some argued the sprouting of ISV would represent type II vasculogenesis (Childs et al., 2002), these two processes are well accepted to represent early embryonic angiogenesis.

Traditional assay to examine zebrafish angiogenesis includes alkaline-phosphatase (AP) staining of endothelial cells and whole-mount in situ hybridization of genes associated with vascular development such as *fli1*, *flk1*, *flt4*, *efnb2a* etc. Although in situ hybridization could provide more specific information such as artery or vein specification (Lawson and Weinstein, 2002a), these methods preclude direct and real-time visualization of the vasculature. Also, it takes days to complete staining protocols. These shortcomings have limited the application of zebrafish model until the recent advancement in zebrafish transgenesis and the availability of tissue-specific stable fluorescent reporter transgenic lines. With the use of fluorescent report transgenic zebrafish line such as Tg(*fli1*:*egfp*) (Lawson and Weinstein, 2002b) or Tg(*flk1*:*egfp*) (Jin et al., 2005), embryonic angiogenesis could be easily monitored real-time under fluorescent microscope. Figure 1 demonstrates the development of ISV and SIV at 48 and 72 hpf with Tg(*flk1*:*egfp*) and Tg(*fli1*:*egfp*).

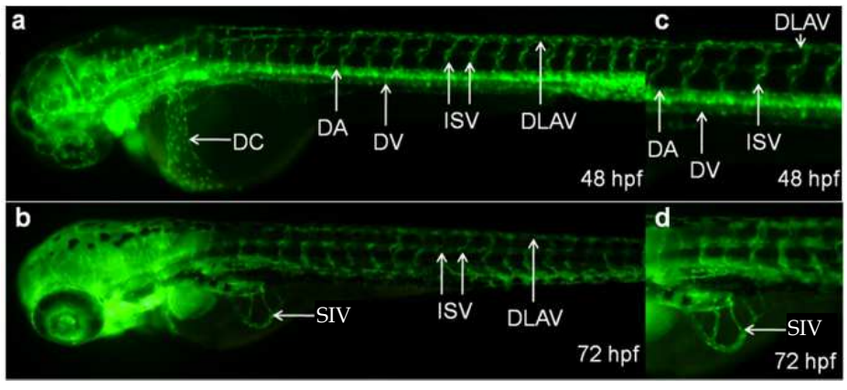


Fig. 1. Endothelial fluorescent transgenic zebrafish embryos showing vascular development at (a, c) 48 and (b, d) 72 hpf. (a, c): Tg(*flk1*:*egfp*); (b, d): Tg(*fli1*:*egfp*). DC: Duct of Curvier; DA: Dorsal aorta; DLAV: dorsal longitudinal anastomotic vessels; DV: Dorsal vein; ISV: Inter-segmental vessel; SIV: sub-intestinal vessels.

3. Modeling tumor angiogenesis in zebrafish

3.1 Gene regulation of zebrafish angiogenesis

While angiogenesis is important for tumor growth and metastasis (Folkman, 2002), the precise mechanism and regulation of tumor angiogenesis remains unclear. Therefore, understanding angiogenesis during normal embryonic development might provide insight into how this process would be perturbed during tumor growth. Previous studies have demonstrated that genes that are involved in tumor angiogenesis such as *galectin-1* (Thijssen et al., 2006), *CXCR7* (Miao et al., 2007), *angiomodulin* (Hooper et al., 2009) and *PDGFR-β/B-Raf* (Murphy et al., 2010) may also play a role in embryonic angiogenesis. The zebrafish is unique in this respect because the circulatory system is dispensable during the first few days of embryonic development, enabling study of genes by specific knock-down that is otherwise lethal in the mammalian system.

3.2 Survivin and zebrafish angiogenesis

We have previously identified zebrafish survivin-1 (Ma et al., 2007a) as an important regulator of embryonic angiogenesis. Survivin exerts its effect through anti-apoptosis and interaction with VEGF receptor kinase pathway. Survivin is the smallest member of the inhibitor of apoptosis (IAP) gene family with a single Baculovirus IAP Repeat (BIR) domain and an extended –COOH terminal α-helical coiled coil (Altieri, 2004). While it is not expressed in most normal adult tissues, survivin is highly expressed in solid and hematological malignancies, where it has been linked to tumor angiogenesis and represented a potential target for anti-cancer therapy (Graaf et al., 1998; Altieri, 2003). During human and murine embryonic development, survivin is ubiquitously expressed (Adida et al., 1998). However, homozygous knock-out of *survivin* in mouse ES cells results in disrupted microtubule formation and polyploidy as well as early embryonic fatality, precluding characterization of its functions during murine development (Uren et al., 2000) and therefore zebrafish embryo was considered an alternative embryonic model.

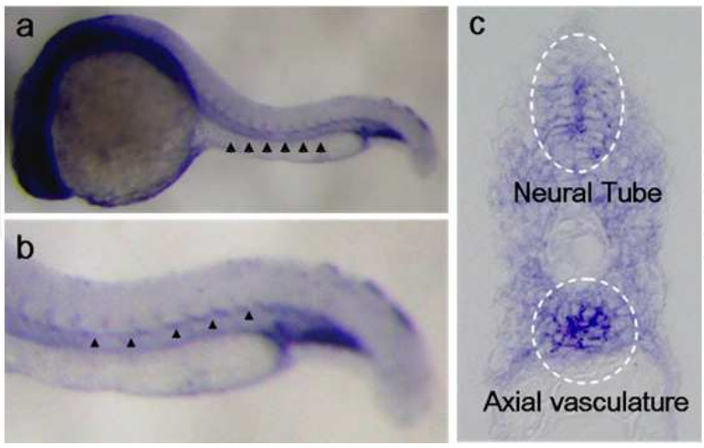


Fig. 2. Expression of *survivin-1* in zebrafish embryo as shown by ISH at 26 hpf. (a,b) Lateral view of whole-mount ISH and black arrowheads denote expression along axial vasculatures. (c) Transverse section of whole-mount ISH showing expression at neural tube and axial vasculatures. Adopted and modified from figure originally published in Ma et al 2007a (with permission).

In zebrafish embryos, *survivin* gene is duplicated into *survivin-1* and *survivin-2*. During embryonic development, *survivin-1* and *survivin-2* are differentially expressed with distinctive functions in the vasculature and hematopoietic tissues (Ma et al., 2007a; Ma et al., 2009). Both survivin-1 and survivin-2 share a highly homologous functional BIR-domain and similar functions at cellular level. Therefore, the distinctive roles of survivin-1 and survivin-2 during embryonic development may be related to a large extent to their difference in spatial expression (Ma et al., 2009). In particular, *survivin-1* predominantly expressed along the neural tube and axial vasculature at 26 hpf (Figure 2). Knock-down of *survivin-1* with anti-sense morpholino gives rise to defective angiogenesis as shown by defective spouting of ISV as well as SIV (Figure 3). Vasculogenesis, demonstrated by the formation of axial vasculatures, was not affected.

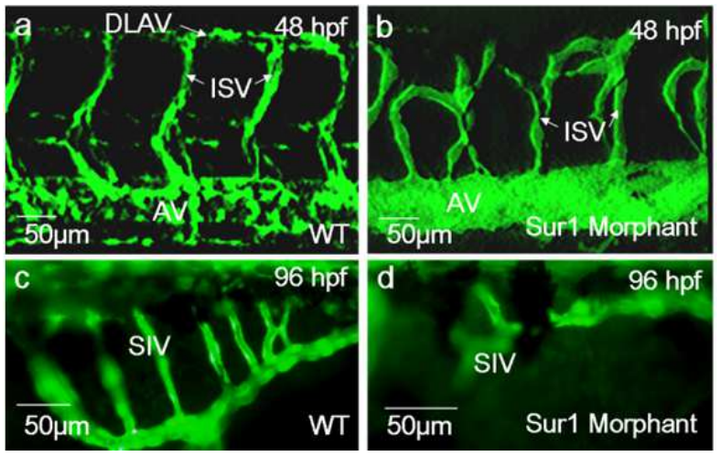


Fig. 3. Effect of *survivin-1* knock-down on zebrafish embryonic angiogenesis. (a, b): Confocal microscopy of *Tg(fli1:egpf)* embryos at 48 hpf either (a) uninjected or (b) injected with *survivin-1* morpholino (MO). Noted the defective sprouting of ISV and the failure to form dorsal longitudinal anastomotic vessels (DLAV) in *survivin-1* (Sur1) morphant. AV: Axial vasculatures. (c, d): *Tg(fli1:egfp)* embryos at 96 hpf showing failure to develop the SIV in Sur1 morphant. Adopted and modified from figure originally published in Ma et al 2007a (with permission).

In vitro and tumorigenesis studies have shown that survivin mediates the angiogenic effects of VEGF (Tran et al., 1999; Mesri et al., 2001; Beierle et al., 2005). In zebrafish embryos, VEGF signaling is also important for angiogenesis. The *schwentine* mutant with defective VEGFR tyrosine kinase, flk1 (Habeck et al., 2002) has perturbed angiogenesis. In addition, phospholipase C-γ (*plc-γ*) mutant (*y10*) (Lawson et al., 2003) as well as knock-down morphant (Ma et al., 2007b) also exhibit specific defects in angiogenesis. VEGF induces ectopic angiogenesis and up-regulates *survivin-1* mRNA expression (Figure 4a-c), suggesting that survivin-1 may mediate the angiogenic effect of VEGF. For instance, we only detect modest apoptotic TUNEL staining in the axial vasculature of *survivin-1* morphant (Figure 4d, e) but not a direct causal link between increased apoptosis and the angiogenesis defect. VEGF might prevent apoptosis (Gupta et al., 1999) and VEGF inhibitors exert pro-apoptotic effect on endothelial cells (reviewed by Epstein, 2007). While apoptotic signal was readily detected along the neural tube of *survivin-1* morphant (Figure 4d, e), survivin-1 might exert its anti-apoptotic effect in a non-cell autonomous fashion downstream of VEGF, regulating the

signaling cues for angioblasts to migrate from aorta to the dorsal aspect of the neural tube and to the inter-phase between notochord and the somites before ISV sprouting (Childs et al., 2002).

3.3 Zebrafish xenograft model of tumor angiogenesis

Recently, zebrafish xenograft models have been developed through xenotransplantation of human primary tumor cells or cancer cell lines into yolk sac of 48 hpf zebrafish embryos (Lee LM et al., 2005; Haldi et al., 2006; Topczewska et al., 2006; Nicoli et al., 2007; Marques et al., 2009). Without a functional immune system at this early embryonic stage, immuno-suppression is not needed. The experimental procedures of transplanting fluorescent labeled human cancer cells into perivitelline space of 48 hpf zebrafish embryos was subsequently published (Nocoli and Presta, 2007). In these models, cancer cells were shown to be engrafted into the yolk sac with proliferation and migration. More importantly, angiogenesis were induced in SIV with infiltration of blood vessels into the cancer mass. Combining with fluorescent reporter transgenic lines, these models serve as a promising platform to study the biology of tumor angiogenesis and its microenvironment including hypoxia (Lee SL et al., 2009) and LIM domain kinase 1 and 2 (Vlecken and Bagowski, 2009).

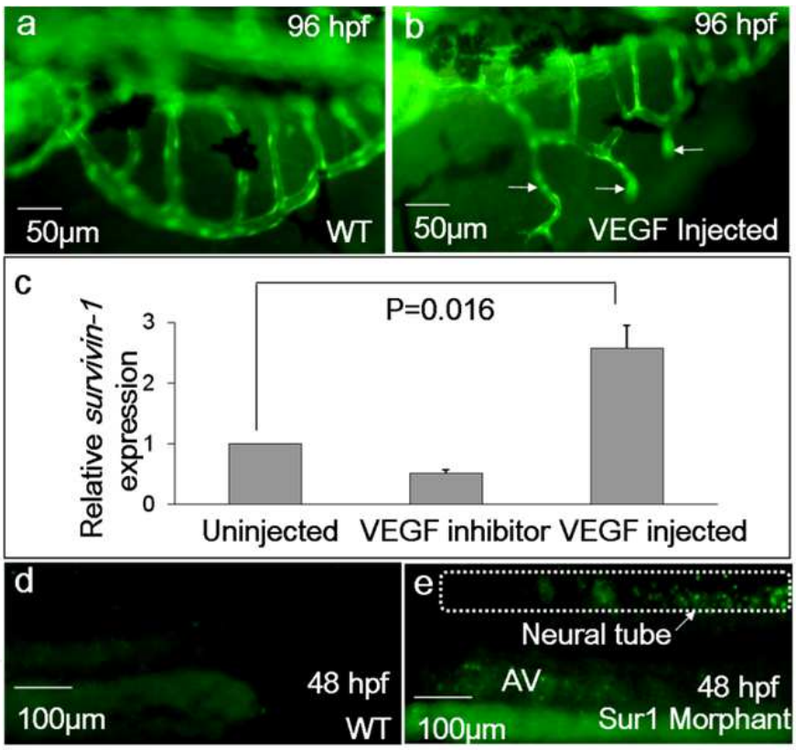


Fig. 4. Survivin-1 interact with VEGF signaling and exert anti-apoptotic activity during zebrafish embryonic angiogenesis. (a, b): Microscopy of *Tg(fli1:egpf)* embryos at 96 hpf either (a) uninjected or (b) injected with human VEGF (2 ng) protein, which induces ectopic angiogenesis (white arrows). (c): relative expression of *survivin-1* mRNA measured by quantitative RT-PCR. (d, e): Whole-mount TUNEL assay in embryos injected with either (e) random sequence or (b) Sur1 MO, which shows positive staining in the area of developing neural tube and at the vicinity of the axial vasculatures (AV) in Sur1 morphant. Adopted and modified from figure originally published in Ma et al 2007a (with permission).

4. Screening potential therapeutic agents with zebrafish embryos

4.1 Large-scale chemical screening platform

Since angiogenesis is crucial for tumor growth and progression, anti-angiogenic agents have been investigated as potential anti-cancer therapies (Demetri et al., 2002; Cunnigham et al., 2004; Shepherd et al., 2005; Van et., al 2007; Hudes et al., 2007). Chemical screening based on in vivo tumor xenograft models are often limited by the relatively low throughput and long read-out time. In this respect, the zebrafish embryo is uniquely suitable for large-scale chemical screening because of the advantages aforementioned. In particular, using the Tg(*flk1:egfp*) or Tg(*fli1:egfp*) embryos, one could conduct large-scale in vivo screening against chemical libraries in a cost-effective way. To examine their effects on the initiation and regression of angiogenesis, embryos will be exposed to chemicals at different concentrations and developmental stages, either before angiogenesis (12 hpf), or after sprouting of ISV and development of SIV (48 hpf). Chemicals that specifically inhibit ISV and SIV formation after 12 hpf likely inhibit the initiation of angiogenesis and those that affect ISV and SIV after their formation at 48 hpf likely induce vascular regression (Figure 5).

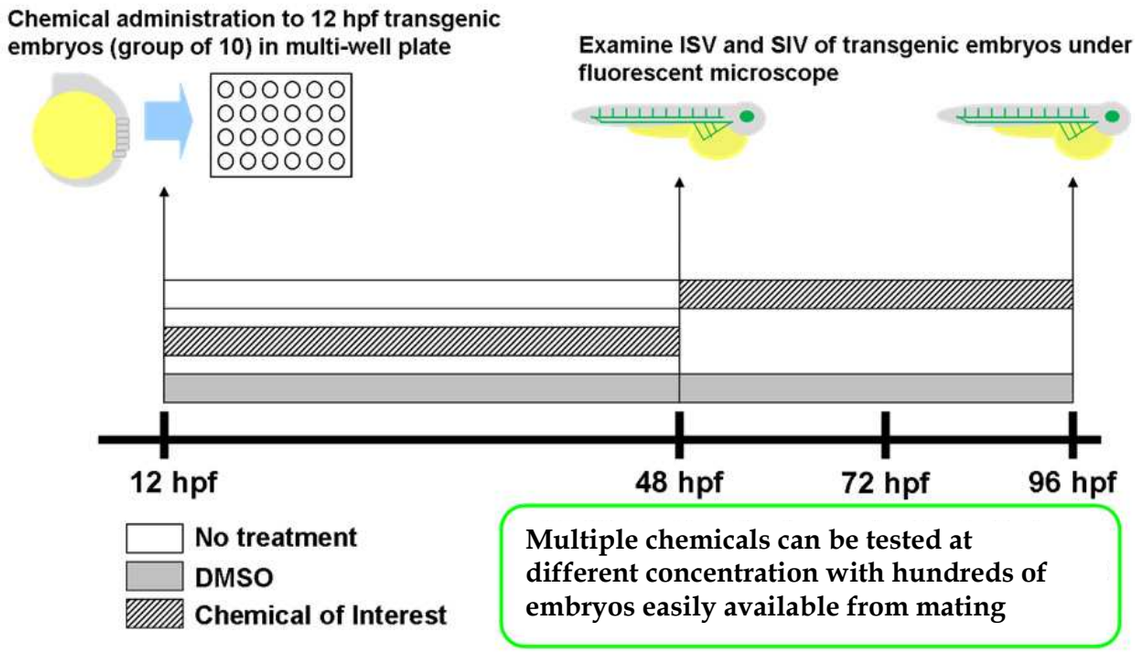


Fig. 5. Cost-effective anti-angiogenic chemicals screening platform with zebrafish embryos.

Both anti-angiogenic mechanisms are considered important component in cancer therapy. This protocol may enable identification of potential anti-angiogenic compounds at high throughput and provide us with novel information about the link between embryonic and tumor angiogenesis. Figure 6 shows the use of Tg(*flk1:egfp*) embryos as a platform to demonstrate anti-angiogenic activity of VEGFR tyrosine kinase inhibitor and anti-cancer drugs (multi-kinase inhibitors) sorafenib and sunitinib.

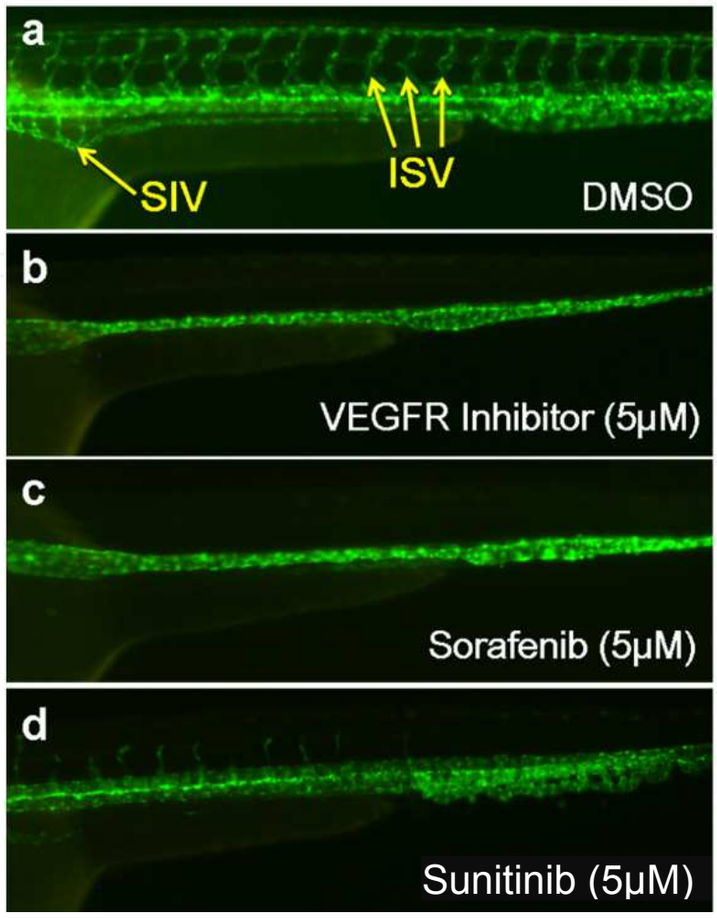


Fig. 6. Demonstration of anti-angiogenic effect of kinase inhibitors with transgenic zebrafish embryos. Microscopy of Tg(*flk1:egfp*) embryos at 48 hpf treated with (a) DMSO, (b) VEGFR tyrosine kinase inhibitor, (c) sorafenib and (d) sunitinib. Treatment with these inhibitors significantly perturbed zebrafish embryonic angiogenesis as shown by development of ISV and SIV.

5. Conclusion

Since angiogenesis is crucial for tumor growth and progression, it may present a potential target for cancer therapy. A number of anti-angiogenic agents targeting at the VEGF signaling pathway are being evaluated and large-scale chemical screening is needed to provide more candidates that can be tested in clinical trials. In this respect, the zebrafish embryos have emerged as a promising model that can shed important lights to the biology of physiological and tumor angiogenesis at whole organism level and allow cost-effective high throughput chemical screening. A number of new genetic modification technologies are now available that can specifically interrogate gene function related to angiogenesis. For instance, artificial endonucleases constructed by fusing non-specific nuclease domain with specific DNA binding domains (Ekker, 2008; Foley et al., 2009a; Foley et al., 2009b; Miller et al., 2011; Cermak et al., 2011; Sander et al., 2011) can now be used to target specific genes from zebrafish genome. An in vivo protein trap mutagenesis system (Clark et al., 2011) is

also available that can simultaneously reveal spatio-temporal protein expression dynamics and assess gene function in zebrafish embryos. These new technologies greatly improve the efficiency of zebrafish genetic modifications and forward genetic screening, making zebrafish a more powerful model organism for angiogenesis.

6. References

Adida C, Crotty PL, Berrebi MD, Diebold J and Altieri DC. (1998). Developmentally regulated expression of the novel cancer anti-apoptosis gene survivin in human and mouse differentiation. *American Journal of Pathology,* Vol.152, No.1, pp.43-49, ISSN 0002-9440

Altieri DC. (2003). Validating survivin as a cancer therapeutic target. *Nature Reviews Cancer,* Vol.3, No.1, pp.46-54, ISSN 1474-175X

Altieri DC. (2004). Molecular circuits of apoptosis regulation and cell division control: The survivin paradigm. *Journal of Cellular Biochemistry,* Vol.92, No.4, pp.656-663, ISSN 0730-2312

Beierle EA, Nagaram A, Dai W, Iyenger M and Chen MK. (2005). VEGF-mediated survivin expression in neuroblastoma cells. *Journal of Surgical Research,* Vol.127, N0.1, pp.21-28, ISSN 0022-4804

Brown KJ, Maynes SF, Bezos A, Maguire DJ, Ford MD and Parish CR. (1996) A novel in vitro assay for human angiogenesis. *Laboratory Invesigation*, Vol.75, No.4, pp.539–555, ISSN 0023-6837

Carmeliet P and Jain RK. (2000). Angiogenesis in cancer and other diseases. *Nature*, Vol.407, No.6810, pp.249-257, ISSN 0028-0836

Cermak T, Doyle EL, Christian M, Wang L, Zhang Y, Schmidt C, Baller JA, Somia NV, Bogdanove AJ and Voytas DF. (2011). Efficient design and assembly of custom TALEN and other TAL effector-based constructs for DNA targeting. *Nucleic Acid Research*, E-published ahead of print, ISSN 0305-1048

Childs S, Chen JN, Garrity DM and Fishman MC. (2002). Patterning of angiogenesis in the zebrafish embryo. *Development, Vol.*129, pp.973–982, ISSN 1011-6370

Clark KJ, Balciunas D, Pogoda HM, Ding Y, Westcot SE, Bedell VM, Greenwood TM, Urban MD, Skuster KJ, Petzold AM, Ni J, Nielsen AL, Patowary A, Scaria V, Sivasubbu S, Xu X, Hammerschmidt M and Ekker SC. (2011). In vivo protein trapping produces a functional expression codex of the vertebrate proteome. *Nature Methods*, Vol.8, No.6, pp.506-512, ISSN 1548-7091

Cunningham D, Humblet Y, Siena S, Khayat D, Bleiberg H, Santoro A, Bets D, Mueser M, Harstrick A, Verslype C, Chau I and Van Cutsem E. (2004). Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer, *New England Journal of Medicine*, Vol.351, No.4, pp.337–345, ISSN 0028-4793

Demetri GD, von Mehren M, Blanke CD, Van den Abbeele AD, Eisenberg B, Roberts PJ, Heinrich MC, Tuveson DA, Singer S, Janicek M, Fletcher JA, Silverman SG, Silberman SL, Capdeville R, Kiese B, Peng B, Dimitrijevic S, Druker BJ, Corless C, Fletcher CD and Joensuu H. (2002). Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. *New England Journal of Medicine*, Vol.347, No.7, pp.472-480, ISSN 0028-4793

Ekker SC. (2008). Zinc finger-based knockout punches for zebrafish genes. *Zebrafish*, Vo.5, No.2, pp.121-123, ISSN 1545-8547

Epstein RJ. (2007). VEGF signaling inhibitors: More pro-apoptotic than anti-angiogenic. *Cancer and Metastasis Reviews*, Vol.26, No.3-4, pp.443-452, ISSN 0167-7659

Foley JE, Maeder ML, Pearlberg J, Joung JK, Peterson RT and Yeh JR. (2009a). Targeted mutagenesis in zebrafish using customized zinc-finger nucleases. . *Nature Protocol,* Vol.4, No.12, pp.1855-1867, ISSN 1754-2189

Foley JE, Yeh JR, Maeder ML, Reyon D, Sander JD, Peterson RT and Joung JK. (2009b). Rapid mutation of endogenous zebrafish genes using zinc finger nucleases made by Oligomerized Pool ENgineering (OPEN). (2009b). *PLoS One,* Vol.4, No.2, e4384, ISSN 1932-6203

Folkman J. (2002). Role of angiogenesis in tumor growth and metastasis. *Seminars in Oncology*, Vol.29, No. 6, Supplement 16, pp.15-18, ISSN 0093-7754

Goishi K and Klagsbrun M. (2004). Vascular endothelial growth factor and its receptors in embryonic zebrafish blood vessel development. *Current Topics of Developmental Biology*, Vol.62, pp.127-152, ISSN 0070-2153

Graaf AO, de Witte T and Jansen JH. (2004). Inhibitor of apoptosis proteins: new therapeutic targets in hematological cancer? *Leukemia, Vol.*18, pp.1751-1759, ISSN 0887-6924

Gupta K, Kshirsagar S, Li W, Gui L, Ramakrishnan S, Gupta P, Law PY and Hebbel RP. (1999). VEGF prevents apoptosis of human microvascular endothelial cells via opposing effects on MAPK/ERK and SAPK/JNL signaling. *Experimental Cell Research*, Vol.247, No.2, pp.495-504, ISSN 0014-4827.

Habeck H, Odenthal J, Walderich B, Maischein HM, Tubingen 2000 screen consortium and Schulte-Merker S. (2002). Analysis of a Zebrafish VEGF Receptor Mutant Reveals Specific Disruption of Angiogenesis. *Current Biology*, Vol.12, No.16, pp.1405-1412, ISSN 0960-9822

Haldi M, Ton C, Seng WL and McGrath P. (2006). Human melanoma cells transplanted into zebrafish proliferates, migrates, produce melanin, from masses and stimulate angiogenesis in zebrafish. *Angiogenesis,* Vol.9, No.3, pp.139-151, ISSN 0969-6970

Hooper AT, Shmelkov SV, Gupta S, Milde T, Bambino K, Gillen K, Goetz M, Chavala S, Baljevic M, Murphy AJ, Valenzuela DM, Gale NW, Thurston G, Yancopoulos GD, Vahdat L,Evans T, and Rafii S. (2009). Angiomodulin is a specific marker of vasculature and regulates vascular endothelial growth factor-A–dependent neoangiogenesis. *Circulation Research*, Vol.105, No.2, pp :201–208, ISSN 0009-7300

Hudes G, Carducci M, Tomczak P, Dutcher J, Figlin R, Kapoor A, Staroslawska E, Sosman J, McDermott D, Bodrogi I, Kovacevic Z, Lesovoy V, Schmidt-Wolf IG, Barbarash O, Gokmen E, O'Toole T, Lustgarten S, Moore L and Motzer RJ. (2007). Temsirolimus, interferon alfa, or both for advanced renal-cell carcinoma. *New England Journal of Medicine*, Vol.356, No.22, pp.2271–2281, ISSN 0028-4793

Isogai S, Horiguchi M and Weinstein BM. (2001). The vascular anatomy of the developing zebrafish: an atlas of embryonic and early larval development. *Developmental Biology, Vol.*230, No.2, pp.278–301, ISSN 0012-1606

Jaffe EA, Nachman RL, Becker CG and Minick CR. (1973). Culture of human endothelial cells derived from umbilical veins. Identification by morphologic and immunologic criteria. *Journal of Clinical Investigation*, vol.52, No.11, pp.2745-2756, ISSN 0021-9738

Jin SW, Beis D, Mitchell T, Chen JN and Stainier DY. (2005). Cellular and molecular analyses of vascular tube and lumen formation in zebrafish. *Development,* Vol.132, pp.5199–5209, ISSN 1011-6370

Jung SP, Siegrist B, Wade MR, Anthoony CT and Woltering EA. (2001). Inhibition of human angiogenesis with heparin and hydrocortisone. *Angiogenesis*, Vol.4, No.3, pp.175-186, ISSN 0969-6970

Lawson ND and Weinstein BM. (2002a). Arteries and veins: Making difference with zebrafish. *Nature Reviews Genetics*, Vol.3, pp.674-682, ISSN 1471-0056

Lawson ND and Weinstein BM. (2002b). In vivo imaging of embryonic vascular development using transgenic zebrafish. *Developmental Biology*, Vol.248, No.2, pp.307-318, ISSN 0012-1606

Lawson ND, Mugford JW, Diamond BA and Weinstein BM. (2003). Phospholipase C gamma-1 is required downstream of vascular endothelial growth factor during arterial development. *Genes and Development,* Vol.17, No.11, pp.1346-51, ISSN 0890-9369

Lee LM, Seftor EA, Bonde G, Cornell RA and Hendrix MJ. (2005). The fate of human malignant melanoma cells transplanted into zebrafish embryos: assessment of migration and cell division in the absence of tumor formation. *Developmental Dynamics*, Vol.233, No.4, pp.1560-1570, ISSN 1097-0177

Lee SL, Rouhi P, Jensen LD, Zhang D, Hauptmann G, Ingham P and Cao Y. (2009). Hypoxia-induced pathological angiogenesis mediates tumor cell dissemination, invasion, and metastasis in zebrafish tumor model. *Proceedings of the National Academy of Science of the United States of America*, Vol.106, No.46, pp.19485-19490, ISSN 0027-8424

Leung T, Chen H, Stauffer AM, Giger KE, Sinha S, Horstick EJ, Humbert JE, Hansen CA and Robishaw JD. (2006). Zebrafish G protein gamma2 is required for VEGF signaling during angiogenesis. *Blood*, Vol.108, No.1, pp.160-166, ISSN 0006-4971

Liang D, Xu X, Chin AJ, Balasubramaniyan NV, Teo MA, Lam TJ, Weinberg ES and Ge R. (1998). Cloning and characterization of vascular endothelial growth factor (VEGF) from zebrafish, Danio rerio. *Biochimica et Biophysica Acta*, Vol.1397, No.1, pp.14-20, ISSN 0167-4781

Ma AC, Lin R, Chan PK, Leung JC, Chan LY, Meng A, Verfaillie CM, Liang R and Leung AY. (2007a). The role of survivin in angiogenesis during zebrafish embryonic development. BMC *Developmental Biology*, Vol.7, No.50. ISSN: 1471-213X

Ma AC, Liang R and Leung AY. (2007b). The role of phospholipase C gamma 1 in primitive hematopoiesis during zebrafish development. *Experimental Hematology,* Vol.35, No.3, pp.368-373, ISSN 0301-472X

Ma AC, Chung MI, Liang R and Leung AY. (2009). The role of survivin2 in primitive hematopoiesis during zebrafish development. *Leukemia,* Vol.23, No.4, pp.712-720, ISSN 0887-6924

Mesri M, Morales-Ruiz M, Ackermann EJ, Bennett CF, Pober JS, Sessa WC and Altieri DC. (2001). Suppression of vascular endothelial growth factor-mediated endothelial cell protection by survivin targeting. *American Journal of Pathology,* Vol.158, No.5, pp.1757-1765, ISSN 0002-9440

Miao Z, Luker KE, Summers BC, Berahovich R, Bhojani MS, Rehemtulla A, Kleer CG, Essner JJ, Nasevicius A, Luker GD, Howard MC and Schall TJ. (2007). CXCR7 (RDC1)

promotes breast and lung tumor growth in vivo and is expressed on tumor-associated vasculature. *Proceedings of the National Academy of Science of the United States of America*, Vol.104, No.40, pp.15735-15740, ISSN 0027-8424

Miller JC, Tan S, Qiao G, Barlow KA, Wang J, Xia DF, Meng X, Paschon DE, Leung E, Hinkley SJ, Dulay GP, Hua KL, Ankoudinova I, Cost GJ, Urnov FD, Zhang HS, Holmes MC, Zhang L, Gregory PD and Rebar EJ. (2011). A TALE nuclease architecture for efficient genome editing. *Nature Biotechnology*, Vol.29, No.2, pp.143-148, ISSN 1087-0156

Murphy EA, Shields DJ, Stoletov K, Dneprovskaia E, McElroy M, Greenberg JI, Lindquist J, Acevedo LM, Anand S, Majeti BK, Tsigelny I, Salsanha A, Waish B, Hoffman RM, Bouvet M, Klemke RL, Vogt PK, Arnold L, Wrasidlo W and Cheresh DA. (2010). Disruption of angiogenesis and tumor growth with an orally active drug that stabilizes the inactive state of PDGFRβ/BRAF. *Proceedings of the National Academy of Science of the United States of America*, Vol.107, No.9, pp.4299–4304, ISSN 0027-8424

Nicoli S, Ribatti D, Cotelli F and Presta M. (2007). Mammalian tumor xenograft induce neovascularization in zebrafish embryos. *Cancer Research*, Vol.67, No.7, pp.2927-2931, ISSN 1578-3445

Nicola S and Presta M. (2007). The zebrafish/tumor xenograft angiogenesis assay. *Nature Protocol*, Vol.2, No.11, pp.2918-2923, ISSN 1754-2189

Sander JD, Cade L, Khayter C, Reyon D, Peterson RT, Joung JK and Yeh JR. (2011). Efficient targeted gene modification in zebrafish using engineered TALE nucleases. *Nature Biotechnology*, in press, ISSN 1087-0156

Siekmann AF and Lawson ND. (2007). Notch signalling limits angiogenic cell behaviour in developing zebrafish arteries. *Nature*, Vol.445, pp.781-784, ISSN 0028-0836

Shepherd FA, Rodrigues Pereira J, Ciuleanu T, Tan EH, Hirsh V, Thongprasert S, Campos D, Maoleekoonpiroj S, Smylie M, Martins R, van Kooten M, Dediu M, Findlay B, Tu D,Johnston D, Bezjak A, Clark G, Santabárbara P and Seymour L. (2005). Erlotinib in previously treated non-small-cell lung cancer, *New England Journal of Medicine*, Vol.353, No.2, pp.123-132, ISSN 0028-4793

Staton CA, Reed MW and Brown NJ. (2009). A critical analysis of current in vitro and in vivo angiogenesis assays. *International Journal of Experimental Pathology*, Vol.90, No.3, pp.195-221, ISSN 1365-2613

Thijssen VL, Postel R, Brandwijk RJ, Dings RP, Nesmelova I, Satijn S, Verhofstad N, Nakabeppu Y, Baum LG, Bakkers J, Mayo KH, Poirier F and Griffioen AW. (2006). Galectin-1 is essential in tumor angiogenesis and is a target for antiangiogenesis therapy. *Proceedings of the National Academy of Science of the United States of America*, Vol.103, No.43, pp.15975-15980, ISSN 0027-8424

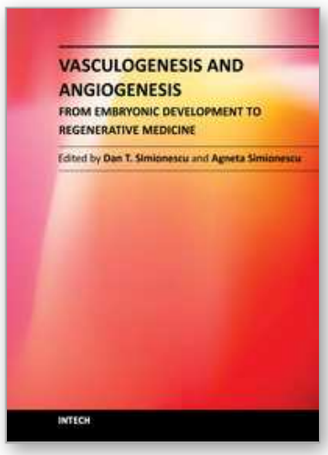
Tran J, Rak J, Sheehan C, Saibil SD, LaCasse E, Korneluk RG and Kerbel RS. (1999). Marked induction of the IAP family antiapoptotic proteins survivin and XIAP by VEGF in vascular endothelial cells. *Biochemical and Biophysical Research Communications,*Vol.264, No.3, pp.781-788, ISSN 0006-291X

Topczewska JM, Postovit LM, Margaryan NV, Sam A, Hess AR, Wheaton WW, Nickoloff BJ, Topczewski J and Hendrix MJ. (2006). Embryonic and tumorigenic pathways converge via nodal signaling: role in melanoma aggressiveness. *Nature Medicine*, Vol.12, No.8, pp.925-932: ISSN 1078-8956

Uren AG, Wong L, Pakusch M, Fowler KJ, Burrows FJ, Vaux DL, Choo KH. (2000). Survivin and the inner centromere protein INCENP show similar cell-cycle localization and gene knockout phenotype. *Current Biology*, Vol.10, No.21, pp.1319-28, ISSN 0960-9822

Van CE, Peeters M, Siena S, Humblet Y, Hendlisz A, B Neyns, Canon JL, Van Laethem JL, Maurel J, Richardson G, Wolf M and Amado RG. (2007). Open-label phase III trial of panitumumab plus best supportive care compared with best supportive care alone in patients with chemotherapy–refractory metastatic colorectal cancer. *Journal of Clinical Oncology*, Vol.25, No.13, pp.1658–1664, ISSN 1743-7563

Vlecken DH and Bagowski CP. (2009). LIMK1 and LIMK2 are important for metastatic behavior and tumor cell-induced angiogenesis of pancreatic cancer cells. *Zebrafish*, Vol.6, No.4, pp.433-439, ISSN 1545-8547



Vasculogenesis and Angiogenesis - from Embryonic Development to Regenerative Medicine

Edited by Dr. Dan Simionescu

ISBN 978-953-307-882-3
Hard cover, 226 pages
Publisher InTech
Published online 07, November, 2011
Published in print edition November, 2011

Vasculogenesis is the process of new blood vessel formation during embryonic development of the cardiovascular system. This is followed by formation of a vascular tree and finally the cardiovascular system with the myriad of blood vessels that nourish all tissues and organs. Angiogenesis, on the other hand is the process by which new blood vessels take shape from existing blood vessels by "sprouting" of endothelial cells thus expanding the vascular tree. Both scenarios are based on activation, migration, proliferation and maturation of unique precursor cells. The study of blood vessel formation is an essential component of embryonic development, congenital malformations, degenerative diseases, inflammation and cancer and thus has widespread appeal to the biomedical field. Moreover, scientists are now harnessing this information for the purpose of building living blood vessel substitutes for replacement of diseased arteries and veins. This book highlights novel advances in the field of vasculogenesis and angiogenesis, including embryogenesis and development, regulation of progenitor cells, cancer and blood vessel regeneration. We consider this book a good initial source of information for graduate students, medical students and scientists interested in the intricacies of blood vessel formation, maturation, disease and replacement.

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen