ARPHA Preprints

PENSOFT

PREPRINT

Posted on 30/12/2020

DOI: https://doi.org/10.3897/arphapreprints.e62570

Molecular characterization of the prostaglandin E receptor subtypes 2a and 4b and their expression patterns during embryogenesis in zebrafish

Wu Hong, Han Yongjun, Chang Hongbo

Not peer-reviewed, not copy-edited manuscript.

Molecular characterization of the prostaglandin E receptor subtypes 2a and 4b and their expression patterns during embryogenesis in zebrafish

Running title: *Ep2a* and *ep4b* expression in zebrafish

Yongjun Han[1], Hongbo Chang[1], Hong Wu[1,2]*

1 Laboratory of Cell Imaging, Henan University of Traditional Chinese Medicine, Zhengzhou 450002, China
2 Institute of Cardiovascular Disease, Henan University of Traditional Chinese Medicine, Zhengzhou 450002, China

*Corresponding author at: Laboratory of Cell Imaging, Henan University of Traditional Chinese Medicine, 6 Dongfeng Rd, Zhengzhou, Henan, 450002, China.
Tel/Fax: +86-371-6090-6297
E-mail address: wuhong@hactcm.edu.cn (H. Wu)

Abstract
The molecular expression profiles of zebrafish *ep2a* and *ep4b* have not been defined to-date. Phylogenetic trees of EP2a and EP4b in zebrafish and other species revealed that human EP4 and zebrafish EP4b were more closely related than EP2a. Zebrafish EP2a is a 281 amino acid protein with high identity to that of human (43%), mouse (44%), rat (43%), dog (44%), cattle (41%), and chicken (41%). Zebrafish EP4b encoded a precursor of 497 amino acids with high amino acid identity to that of mammals, including human (57%), mouse (54%), rat (55%), dog (55%), cattle (56%), and chicken (54%). Whole-mount in situ hybridization revealed that *ep2a* was robustly expressed in the anterior four somites at the 10-somites stages, but was absent in the somites at 19 hpf. It was observed again in the pronephric duct at 24 hpf, in the intermediate cell mass located in the trunk, and in the rostral blood island at 30 hpf. *Ep2a* was also expressed in the notochord at 48 hpf. During somitogenesis, *ep4b* was highly expressed in the eyes, somites, and the trunk neural crest. From 30 to 48 hpf, *ep4b* could be detected in the posterior cardinal vein and the neighboring ICM. From these data, we conclude that *ep2a* and *ep4b* are conserved in vertebrates and that the presence of *ep2a* and *ep4b* transcripts during developmental stages infers their role during early zebrafish larval development. In addition, the variable expression of the two receptor isoforms was strongly suggestive of divergent roles of molecular regulation.

Keywords: *ep2a*; *ep4b*; expression; whole-mount in situ hybridization; zebrafish

Introduction

Prostaglandin E2 (PGE2) is an important arachidonate metabolite that regulates an array of physiological processes in vertebrates, including immune responses (Kutyrev et al. 2017), gastrointestinal function (Takeuchi and Amagase 2018), testicular homeostasis (Rey-Ares et al. 2018), and ovulation (Baker and Van Der Kraak 2019). PGE_2 binds to four specific G-protein-coupled receptors (EP1, EP2, EP3, and EP4) in humans and mice (Ball et al. 2013; Kimple et al. 2013; Zhu et al. 2018). EP1 is coupled to Gq to elevate intracellular Ca^{2+}, EP3 couples to Gi to inhibit adenylyl cyclase (AC) and cyclic AMP (cAMP), and both EP2 and EP4 couple to Gs to stimulate AC and increase cAMP (Samuchiwal et al. 2017; Wang et al. 2010), and have been identified as crucial mediators of PGE_2 in both physiological and pathophysiological processes, including closure of the ductus arteriosus (Sakuma et al. 2018; Yokoyama et al. 2014), bone formation (Graham et al. 2009), ovulation and fertilization (Niringiyumukiza et al. 2018) and salt sensitive hypertension (Kennedy et al. 1999). Knockdown of EP2a results in smaller embryonic livers in zebrafish (Nissim et al. 2014). In medaka, impairment of the EP4b receptor *in vitro* using a competitive antagonist resulted in anovulation (Fujimori et al. 2012).

Though both EP2 and EP4 were identified in mammals (Kershaw-Young et al. 2009; Kowalewski et al. 2008; Reitmair et al. 2010) and chickens (Kwok et al. 2008), their expression has not been investigated in fish. Zebrafish (Danio rerio) are a vertebrate model organism that is widely used for genetic and pharmacological analysis of embryogenesis due to their high fecundity and translucency (Akhter et al. 2016; Ellertsdottir et al. 2010). In addition, a number of disease models have been developed in zebrafish that can be combined with *in vivo* imaging approaches to monitor specific pathological processes, including cardiovascular disease and cancer metastasis (Bournele and Beis 2016; Tulotta et al. 2016). In this regard, it has been shown that prostaglandin receptors play a key role in zebrafish development, including ovulation and T cell precursor development (Baker and Van Der Kraak 2019; Villablanca et al. 2007). However, the expression of zebrafish *ep2a* and *ep4b* has not been systematically defined in the literature.

Here, we report the expression patterns of *ep2a* and *ep4b* during zebrafish embryogenesis. We show that *ep2a* is robustly expressed in the somites, the pronephric duct, ICM, and notochord, whilst *ep4b* is highly expressed in the somites, the trunk neural crest, the posterior cardinal vein and the neighboring ICM. These data reveal important information regarding the expressional characteristics of zebrafish *ep2a* and *ep4b* during developmental stages. Differences in the expression of these transcripts during development indicate differential molecular regulatory patterns.

Materials and methods

Zebrafish models

Embryos of AB wild type zebrafish were raised and staged as described by Kimmel et al (Kimmel et al. 1995). Embryos were maintained in E3 solution at 28.5 °C with 0.003% 1-phenyl-2-thiourea (Sigma) to inhibit pigmentation. Embryos were staged according to somite number or hours post-fertilization (hpf).

Cloning of ep2a and ep4b

Total RNA was extracted from zebrafish embryos at 0.5-24 hpf stages using commercially

available RNeasy mini-kits (Qiagen) according to manufacturer's protocols. First-strand cDNA was generated from normalized input RNA using random hexamers and commercial Superscript II kits (Invitrogen). RT-PCRs were performed with specific primer pairs: *ep2a* (846 bp fragment, forward: 5'-CGGATCCGATGGGCACTGAAAATGGGACCTGTA-3', BamHI; reverse: 5'-CATCGATTTATTTTCTTTCGTTACAATCACAT-3', ClaI), *ep4b* (1494 bp fragment, forward: 5'-CATCGATATGAAACGCACGTGTGGAAAGTATG-3', ClaI; reverse: 5'- CGGAATTCATATGGTTCTCTCTTGAAAGCTC-3', EcoRI). PCR parameters were as follows: 95 °C for 3 min, (95 °C for 30 s, 60 °C for 30 s, 72 °C for 30 s) x 35, 72 °C for 5 min. Full-length PCR products were cloned into the pGEM-T easy vector (Promega) and used for antisense digoxigenin labeled probe synthesis with SP6 RNA polymerase.

Sequence alignment and phylogenetic analyses

EP2 and EP4 orthologs were retrieved from GenBank and included Danio rerio EP2a (Accession Nos.: NM_200635.1), homo sapiens EP2 (NM_000956.1), Mus musculus EP2 (NM_008964.1), Canis lupus EP2 (NM_001003170.1), Rat EP2 (NM_031088.1), Bos taurus EP2 (AF539402.1), Gallus EP2 (EF200120.1), Dario rerio EP4b (NM_001128367.1), Homo sapiens EP4 (NM_000958.1), Mus musculus EP4 (NM_001136079.1), Rat EP4 (NM_032076.1), Canis lupus EP4 (NM_001003054.1), Bos taurus EP4 (AF539403.1), and Gallus EP4 (EF200121.1). Alignments were performed based on derived amino acid sequences using MUSCLE in MEGA version 5.

A phylogenetic tree was constructed using the sequences of zebrafish, human, mouse, rat, dog, cattle, and chicken EP2 and EP4 via the "AllAll program". Analysis was performed using the Computational Server at Eidgenössische Technische Hochschule Zürich (ETHZ) (http://www.cbrg.ethz.ch/services/AllAll).

Whole mount in situ hybridization

Antisense probes for *ep2a* and *ep4b* were synthesized using DIG RNA labeling kit (Roche, US). Standard procedures were performed as per the manufacturer's recommendations. Whole mount *in situ* hybridizations was performed as described by Thisse et al (Thisse and Thisse 2014). Embryos were imaged on a Zeiss confocal microscope. In situ experiments were performed on a minimum of three independent occasions using 20 embryos.

Results

Sequence alignment of EP2a from various vertebrate species

Full-length cDNAs for zebrafish *ep2a* and *ep4b* were cloned and the sequences were verified by blast searches. Full-length cDNA of zebrafish *ep2a* was 2366 bp (GenBank accession No: NM_200635.1), encoded a 281 amino acid protein (Tsuge et al. 2013). As expected, zebrafish EP2a exhibited high amino acid identity to that of human (Homo sapiens) (43%), mouse (Mus musculus) (44%), rat (Rattus norvegicus) (43%), dog (Canis lupus familiaris) (44%), cattle (Bos taurus) (41%), and chicken (Gallus gallus) (41%) E2a. The protein possessed seven putative transmembrane domains. In contrast, the amino acid identity of EP2 significantly differed between zebrafish and other species. In the ORF region of EP2, three highly variable regions were identified, located in intracellular loop 1, extracellular loop 2, and intracellular loop 3 (Figure 1).

Phylogenetic tree of EP2a in different species

As shown in Figure 2, a close distance between chicken EP2 and zebrafish EP2a was observed. Interestingly, the distance between human EP2 and zebrafish EP2a was less close. Analysis of the phylogenetic tree indicated that the zebrafish EP2a receptor branched from its ancestor. Zebrafish EP2a receptors similarly diverged from their ancestors. This branching may have occurred due to the different ligand recognition properties of the EP2 receptors.

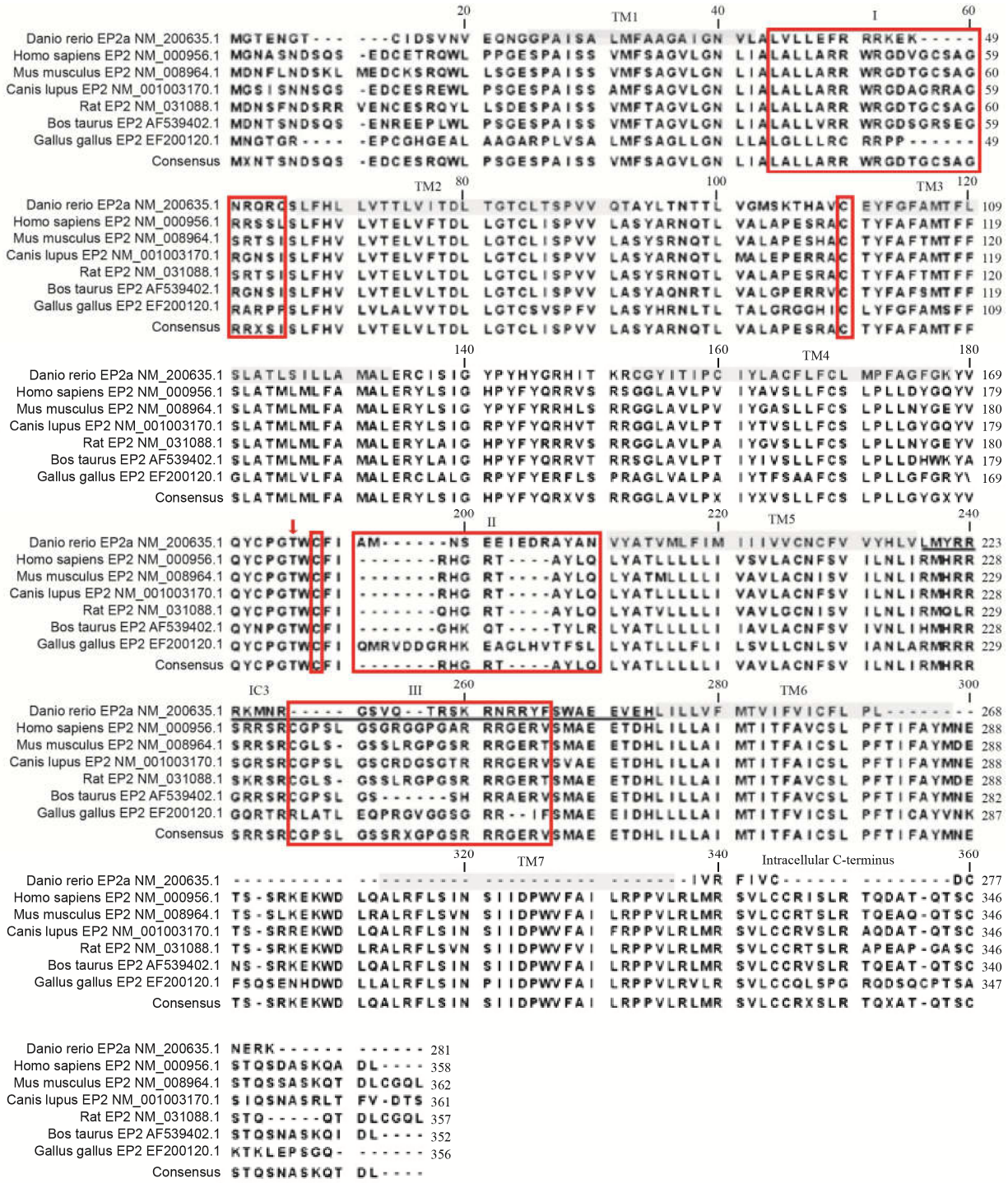

Fig 1. Sequence alignment of zebrafish EP2a (Danio rerio EP2a: NM_200635.1) to human

(Homo sapiens EP2: NM_000956.1), mouse (Mus musculus EP2: NM_008964.1), rat (Rat EP2: NM_031088.1), dog (Canis lupus EP2: NM_001003170.1), cattle (Bos taurus EP2: AF539402.1) and chicken (Gallus gallus EP2: EF200120.1). The seven putative transmembrane domains are shaded and labeled. Sequences underlined and in bold represent the third intracellular loop (IC3). Three highly variable regions (I, II, and III) between zebrafish and other species are boxed and labeled. Arrow heads indicate the conserved threonine. Two cysteine residues forming disulphide bonds are boxed. Dots indicate amino acids that are not present within the sequence.

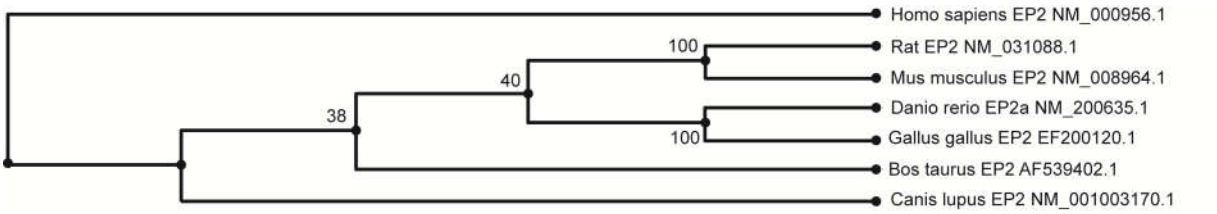

Fig 2. Phylogenetic tree of EP2 across different species constructed using the Neighbor-joining method. An evolutionary relationship of EP2 in different species, including human, mouse, rat, dog, cattle, chicken and zebrafish were observed. Numbers adjacent to the branch points indicate bootstrap values.

Sequence alignment of EP4b proteins from various vertebrate species

The full-length cDNA of zebrafish *ep4b* was 1494 bp (Genbank accession NM_001128367.1) encoding a protein of 497 amino acids (Figure 3). Zebrafish EP4b showed high sequence homology to human (57%), mouse (54%), rat (55%), dog (55%), cattle (56%), and chicken (54%) EP4 (Figure 3). Despite the high homology, the 3rd intracellular loop and intracellular C-terminus significantly differed between zebrafish and other species (Figure 3).

Phylogenetic tree of EP4b across different species

Figure 4 shows the phylogenetic tree of EP4 in zebrafish and other species. The distance between chicken EP4 and zebrafish EP4b was close. Interestingly, human EP4 and zebrafish EP4b were also closely related compared to EP2a. ONO-AE3-208, an antagonist for human EP4, effectively blocks zebrafish EP4b activity (Tsuge et al. 2013). Thus, the response of each zebrafish EP4 receptor to these compounds was closely related to their structural conservation with the human receptor.

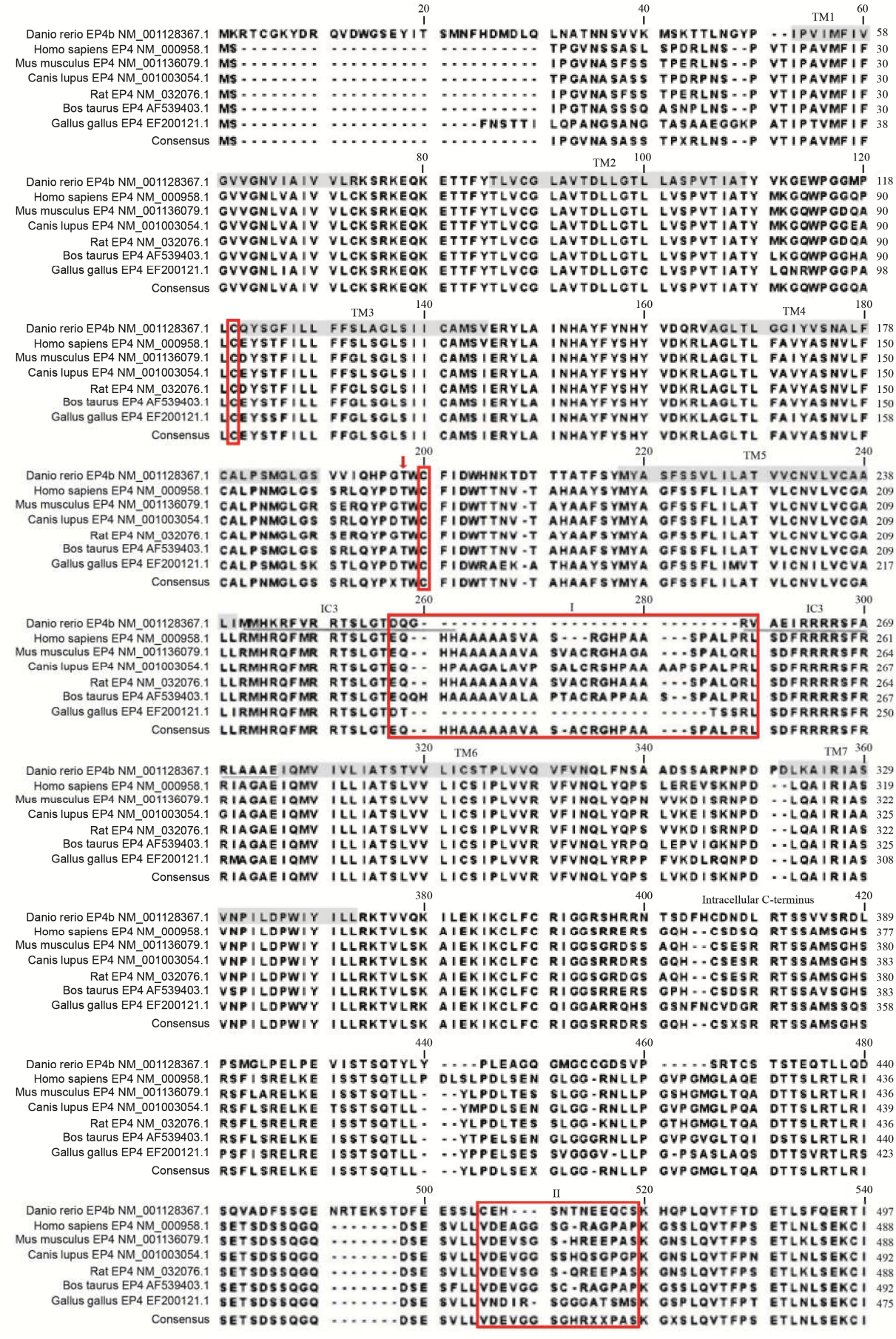

Fig 3. Sequence alignment of zebrafish EP4b (Dario rerio EP4b: NM_001128367.1) to that of human (Homo sapiens EP4: NM_000958.1), mouse (Mus musculus EP4: NM_001136079.1), rat (Rat EP4: NM_032076.1), dog (Canis lupus EP4: NM_001003054.1), cattle (Bos taurus EP4: AF539403.1), and chicken EP4 (Gallus EP4: EF200121.1). The seven putative transmembrane domains are shaded and labeled. Sequences underlined and in bold represent the third intracellular loop (IC3). Two highly variable regions (I and II) of EP4 between zebrafish and other species are boxed. The two cysteine residues proposed to form disulphide bonds are also boxed. Arrow heads indicate the conserved threonine residue of EP4. Dots indicate amino acids that are absent within the sequence.

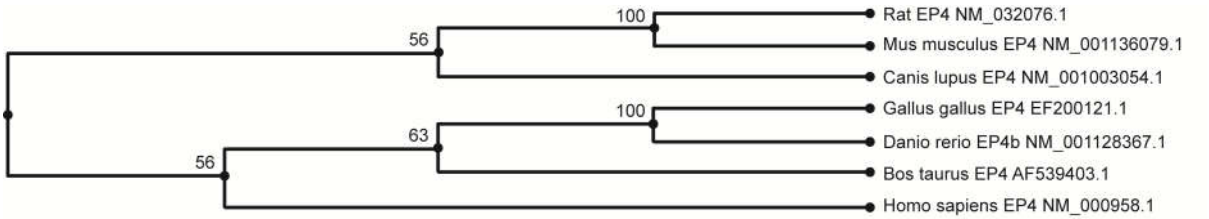

Fig 4. Phylogenetic tree of EP4 across the indicated species. The phylogenetic tree was constructed using the Neighbor-joining method. Evolutionary relationships of EP4 in different species, including human, mouse, rat, dog, cattle, chicken and zebrafish. Numbers adjacent to the branch points indicate bootstrap values.

Expression of zebrafish ep2a during early embryogenesis

Whole mount in situ hybridization was used to determine the temporal and spatial expression patterns of *ep2a*. At the 10-somites stage (14 hpf), robust *ep2a* expression was observed in the anterior four somites (Figure 5A). Interestingly, at 19 hpf, *ep2a* could no longer be detected in the somites, although its expression was diffuse in the anterior region of the embryo (Figure 5B). At 24 hpf, *ep2a* expression was observed in the pronephric duct, and diffusely in the posterior region of the trunk (Figure 5C). At 30 hpf, *ep2a* was maintained in the ICM of the trunk and in the rostral blood island (Figure 5D). At 48 hpf, *ep2a* was strongly expressed in the notochord, but weakly expressed in the blood (Figure 5E).

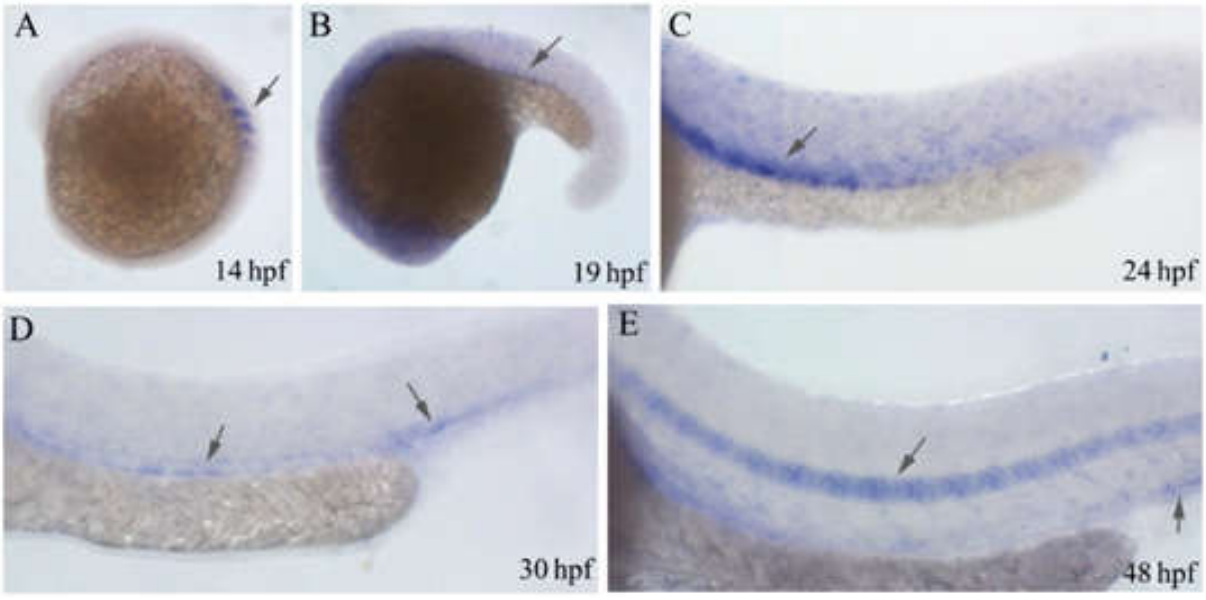

Fig 5. *Ep2a* expression during zebrafish development. (A) *ep2a* was expressed in the anterior

four somites at 14 hpf. (B) *ep2a* was ubiquitously expressed in the anterior region of the emybros at 19 hpf. (C) At 24 hpf, *ep2a* expression was observed in the pronephric duct and diffusely in the posterior region of the trunk. (D) At 30 hpf, *ep2a* expression was maintained in the ICM located in the trunk and in the rostral blood island. (E) At 48 hpf, *ep2a* was strongly expressed in the notochord, but weakly expressed in the blood. In (A) embryos are orientated in the lateral view with the anterior side up and the dorsal side to the right. Embryos in (B-E) are orientated in the lateral view with the anterior to the left and the dorsal side up. *ep2a* expression is indicated with arrows.

Zebrafish ep4b expression during early embryogenesis

Whole mount in situ hybridization was used to investigate the temporal and spatial expression pattern of *ep4b*. During the shield stage, *ep4b* was present in the germ ring, showing peak expression in the embryonic shield (Figure 6A). At the end of gastrulation, *ep4b* became more localized to the posterior region of the embryo (Figure 6B). The dorsal view showed that the expression was arranged in two sides (ellipse) of the prechordal plate hypoblast (Figure 6C), in which the cells contribute to the posterior trunk, somites, and neural (Kimmel et al. 1995). During somitogenesis, *ep4b* was robustly expressed in the eyes, somites, and the trunk neural crest (Figure 6D and E). At 24 hpf, *ep4b* remained highly expressed in the somites and the trunk neural crest (Figure 6F). *Ep4b* expression in the trunk neural crest continued to the 30 hpf stage (Figure 6G). From 30 to 48 hpf, *ep4b* could be detected in the posterior cardinal vein and the neighboring ICM (Figure 6G and H), as reported for *ptger4a* (Baker and Van Der Kraak 2019).

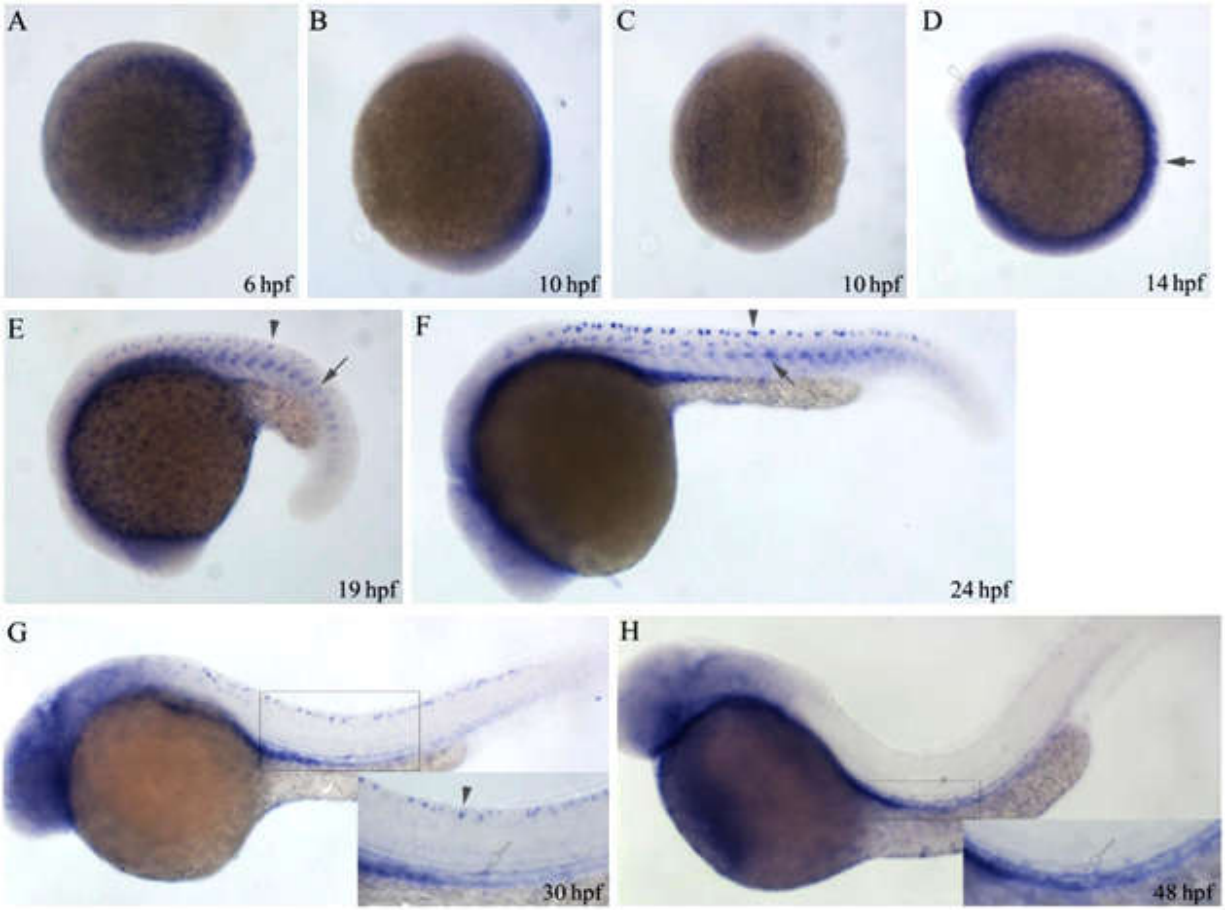

Fig 6. *Ep4b* expression during zebrafish development. (A) Embryonic animal polar diagrams at 6 hpf show that *ep4b* displayed a graded pattern of expression in the embryonic ring, with

the highest expression levels observed in the embryonic shield. (B) Lateral views (anterior side up and the dorsal to the right) of the embryo show that *ep4b* was expressed in the posterior region of the emybro at 10 hpf. (C) Dorsal view (anterior side up) showing that the expression was arranged in two sides (ellipse) of the prechordal plate hypoblast. (D) Lateral views (anterior side up and the dorsal to the right) of the embryo at 14 hpf show that *ep4b* was expressed in the somites (black arrows), and the eyes (white arrowheads). (E-H) Lateral views (anterior to the right and the dorsal side up) of the embryos. At 19 hpf (E) and 24 hpf (F), black arrows in (E and F) indicate that *ep4b* was expressed in the somites, the trunk neural crest (black arrowhead). (G and H) show that *ep4b* was expressed in the trunk neural crest (black arrowhead) at 30 hpf (G), in the posterior cardinal vein, and in the neighboring ICM (white arrow) at 30 hpf and at 48 hpf (H). Insets represent magnification of the boxes in (G and H).

Discussion

In this study, we show that *ep2a* is robustly expressed in the anterior four somites at 14 hpf, but is absent in the somites at 19 hpf. At later stages, *ep2a* could be observed in the pronephric duct, ICM, and in the rostral blood island between 24 to 30 hpf. Robust expression was observed in the notochord at 48 hpf. Interestingly, the expression of *ep2a* and *ep4b* differed during zebrafish development. During somitogenesis, *ep4b* was present in the eyes, somites, and the trunk neural crest. From 30 to 48 hpf, *ep4b* was present in the posterior cardinal vein and the neighboring ICM. Variations in the expression of these transcripts during developmental stages suggested different modes of molecular regulation.

Species comparison of EP2 and EP4 showed that EP2a and EP4b showed high homology across mammalian and non-mammalian vertebrate species, including human (43%; 57%, respectively), mouse (44%; 54%, respectively), rat (43%; 55%, respectively), dog (44%; 55%, respectively), cattle (41%; 56%, respectively), and chicken (41%; 54%, respectively), as shown in Figure 1 and 3. Of note, the distance between human EP4 and zebrafish EP4b was close compared to human and zebrafish IP receptors. Interestingly, the distance between human EP4 and zebrafish EP4c was less close (Tsuge et al. 2013). Consistent with these data, ONO-AE1-329, an agonist specifically designed for human EP4, showed high activity against zebrafish EP4b but low activity against the EP4c receptor (Stillman et al. 1998). Thus, zebrafish EP4 receptors and their interaction with these compounds were closely related to the structural conservation of the human receptor. Compared with EP4 and IP clusters, EP2 receptors showed higher differentiation. The distance between human EP2 and zebrafish EP2a was relatively large.

EP2 and EP4 belong to the GPCR superfamily and can be subdivided into rhodopsin receptor members. Both share common characteristics. Phylogenetic trees with bootstrap values for EP2 and EP4 were constructed using the Neighbor-joining method (Figure 2 and 4), and indicated that both EP2 and EP4 are closely related to their corresponding counterparts in mammalian species and chicken. This suggested that the cloned receptors are potential EP2 and EP4 receptors in zebrafish. Two highly conserved cysteines present amongst GPCRs (Stillman et al. 1998) with proposed roles in disulphide bond formation were located as Cys110 and Cys188 in EP2, and Cys122 and Cys200 in EP4 (Figure 1 and 3). A threonine residue that is conserved amongst EP2 and EP4 has also been suggested to be important for ligand binding (Stillman et al. 1998), and was identified in the cloned receptors (Thr186 in EP2 and Thr198 in EP4) (Figure 1 and 3). Despite these similarities, EP2 and EP4 showed several distinct differences. As in mammals (Desai et al. 2000), EP4 was longer than EP2 (497 a.a. vs. 281 a.a.), with the major differences observed in the lengths of the 3rd intracellular loops and intracellular C-termini (Figure 1 and 3). The longer

C-terminus of EP4 possesses a number of putative phosphorylation sites that undergo short term ligand-induced receptor internalization and desensitization. This was suggestive of potential functional differences in signal transduction between EP2 and EP4 in response to their ligands (Davidson and Zon 2004; Sugimoto and Narumiya 2007).

Using whole mount in situ hybridization, *ep2a* and *ep4b* expression were analyzed in the zebrafish embryos. Robust expression of *ep2a* was observed in the anterior four somites at 14 hpf (Figure 5A), indicating its relationship to the development stages of somatic cells. At 24 hpf, *ep2a* expression was observed in the pronephric duct, mainly and in the posterior region of the trunk with diffuse but ubiquitous expression (Figure 5C). At 30 hpf, *ep2a* is expressed in the ICM and is located in the trunk and in the rostral blood island (Figure 5D), whilst definitive hemopoiesis occurred in the DA-PCV joint region, which is equivalent to the AGM region (Villablanca et al. 2007). Similarly, *pges* was expressed in the endothelium, and *cox-2* was expressed in the wall of the aortic arch (Pini et al. 2005). The expression patterns of *ep2a* and *cox-2* and *pges-1* in zebrafish highlight their potential during vascular development and ability to modulate key components of these developmental stages, including VEGF. During somitogenesis, *ep4b* was expressed in the eyes, somites, and the trunk neural crest (Figure 6D and E). At 24 hpf, *ep4b* remained highly expressed in the somites and the trunk neural crest (Figure 6F). In addition, at 30 hpf, *ep4b* was expressed in the trunk neural crest (Figure 6G). These data suggest that *ep4b* plays an important role in the development of somites and the trunk neural crest. From 30 to 48 hpf, *ep4b* expression could be detected in the posterior cardinal vein and the neighboring ICM (Figure 6G and H), and was similar to that observed for *ep4b* (Baker and Van Der Kraak 2019). In addition, *cox-1* and *cox-2* were expressed in the carotid arteries and the vasculature of the pharyngeal arches at 96 hpf (Ishikawa et al. 2007; Pini et al. 2005). This highlights a novel role for the PGE_2 / EP4 axis during vascular development.

Conclusion

In this study, full-length cDNAs for zebrafish *ep2a* and *ep4b* were cloned and their expression patterns were characterized during zebrafish development. *Ep2a* was mainly expressed in the somites, pronephric duct, ICM, and notochord, while *ep4b* was expressed in the somites, the trunk neural crest, and ICM. The differences in expression between *ep2a* and *ep4b* transcripts during these developmental stages highlight divergent modes of molecular regulation during developmental stages.

Conflicts of interest

No conflict of interest to declare.

Acknowledgments

This work was supported by grants from the research nursery project of Henan university of traditional Chinese medicine (Grant No. MP2016-10), the Key projects of Henan Educational Committee (Grant No. 18A360007), the project of National TCM clinical research base (Grant No. 2019JDZX023).

References

Akhter A, Kumagai R, Roy SR, Ii S, Tokumoto M, Hossain B, Wang J, Klangnurak W, Miyazaki T, Tokumoto T (2016) Generation of Transparent Zebrafish with Fluorescent

Ovaries: A Living Visible Model for Reproductive Biology. Zebrafish 13(3): 155-160. https://doi.org/10.1089/zeb.2015.1116
Ball BA, Scoggin KE, Troedsson MH, Squires EL (2013) Characterization of prostaglandin E2 receptors (EP2, EP4) in the horse oviduct. Animal Reproduction Science 142(1-2): 35-41. https://doi.org/10.1016/j.anireprosci.2013.07.009
Desai S, April H, Nwaneshiudu C, Ashby B (2000) Comparison of agonist-induced internalization of the human EP2 and EP4 prostaglandin receptors: role of the carboxyl terminus in EP4 receptor sequestration. Molecular Pharmacology 58(6): 1279-1286. https://doi.org/10.1124/mol.58.6.1279
Ellertsdottir E, Lenard A, Blum Y, Krudewig A, Herwig L, Affolter M, Belting HG (2010) Vascular morphogenesis in the zebrafish embryo. Developmental Biology 341(1): 56-65. https://doi.org/10.1016/j.ydbio.2009.10.035
Fujimori C, Ogiwara K, Hagiwara A, Takahashi T (2012) New evidence for the involvement of prostaglandin receptor EP4b in ovulation of the medaka, Oryzias latipes. Molecular and Cellular Endocrinology 362(1-2): 76-84. https://doi.org/10.1016/j.mce.2012.05.013
Graham S, Gamie Z, Polyzois I, Narvani AA, Tzafetta K, Tsiridis E, Helioti M, Mantalaris A, Tsiridis E (2009) Prostaglandin EP2 and EP4 receptor agonists in bone formation and bone healing: In vivo and in vitro evidence. Expert Opinion on Investigational Drugs 18(6): 746-766. https://doi.org/10.1517/13543780902893051
Ishikawa TO, Griffin KJ, Banerjee U, Herschman HR (2007) The zebrafish genome contains two inducible, functional cyclooxygenase-2 genes. Biochemical and Biophysical Research Communications 352(1): 181-187. https://doi.org/10.1016/j.bbrc.2006.11.007
Kennedy CR, Zhang Y, Brandon S, Guan Y, Coffee K, Funk CD, Magnuson MA, Oates JA, Breyer MD, Breyer RM (1999) Salt-sensitive hypertension and reduced fertility in mice lacking the prostaglandin EP2 receptor. Nature Medicine 5(2): 217-220. https://doi.org/10.1038/5583
Kershaw-Young CM, Khalid M, McGowan MR, Pitsillides AA, Scaramuzzi RJ (2009) The mRNA expression of prostaglandin E receptors EP2 and EP4 and the changes in glycosaminoglycans in the sheep cervix during the estrous cycle. Theriogenology 72(2): 251-261. https://doi.org/10.1016/j.theriogenology.2009.02.018
Kimmel CB, Ballard WW, Kimmel SR, Ullmann B, Schilling TF (1995) Stages of embryonic development of the zebrafish. Dev Dyn 203:253-310.
Kimple ME, Keller MP, Rabaglia MR, Pasker RL, Neuman JC, Truchan NA, Brar HK, Attie AD (2013) Prostaglandin E2 receptor, EP3, is induced in diabetic islets and negatively regulates glucose- and hormone-stimulated insulin secretion. Diabetes 62(6): 1904-1912. https://doi.org/10.2337/db12-0769
Kowalewski MP, Mutembei HM, Hoffmann B (2008) Canine prostaglandin E2 synthase (PGES) and its receptors (EP2 and EP4): expression in the corpus luteum during dioestrus. Animal Reproduction Science 109(1-4): 319-329. https://doi.org/10.1016/j.anireprosci.2007.11.023
Kutyrev IA, Biserova NM, Olennikov DN, Korneva JV, Mazur OE (2017) Prostaglandins E2 and D2-regulators of host immunity in the model parasite Diphyllobothrium dendriticum: An immunocytochemical and biochemical study. Molecular and Biochemical Parasitology 212: 33-45. https://doi.org/10.1016/j.molbiopara.2017.01.006
Kwok AH, Wang Y, Wang CY, Leung FC (2008) Molecular cloning and characterization of chicken prostaglandin E receptor subtypes 2 and 4 (EP2 and EP4). General and Comparative Endocrinology 157(2): 99-106. https://doi.org/10.1016/j.ygcen.2008.04.001
Niringiyumukiza JD, Cai H, Xiang W (2018) Prostaglandin E2 involvement in mammalian female fertility: ovulation, fertilization, embryo development and early implantation. Reproductive Biology and Endocrinology 16(1): 43. https://doi.org/10.1186/s12958-018-

0359-5
Nissim S, Sherwood RI, Wucherpfennig J, Saunders D, Harris JM, Esain V, Carroll KJ, Frechette GM, Kim AJ, Hwang KL, Cutting CC, Elledge S, North TE, Goessling W (2014) Prostaglandin E2 regulates liver versus pancreas cell-fate decisions and endodermal outgrowth. Developmental Cell 28(4): 423-437. https://doi.org/10.1016/j.devcel.2014.01.006
Pini B, Grosser T, Lawson JA, Price TS, Pack MA, FitzGerald GA (2005) Prostaglandin E synthases in zebrafish. Arteriosclerosis, Thrombosis, and Vascular Biology 25(2): 315-320. https://doi.org/10.1161/01.atv.0000152355.97808.10
Reitmair A, Lambrecht NW, Yakubov I, Nieves A, Old D, Donde Y, Dinh D, Burk R, Sachs G, Im WB, Wheeler L (2010) Prostaglandin E2 receptor subtype EP2- and EP4-regulated gene expression profiling in human ciliary smooth muscle cells. Physiological Genomics 42(3): 348-360. https://doi.org/10.1152/physiolgenomics.00012.2010
Rey-Ares V, Rossi SP, Dietrich KG, Kohn FM, Schwarzer JU, Welter H, Frungieri MB, Mayerhofer A (2018) Prostaglandin E2 (PGE2) is a testicular peritubular cell-derived factor involved in human testicular homeostasis. Molecular and Cellular Endocrinology 473: 217-224. https://doi.org/10.1016/j.mce.2018.01.022
Sakuma T, Akaike T, Minamisawa S (2018) Prostaglandin E2 Receptor EP4 Inhibition Contracts Rat Ductus Arteriosus. Circulation Journal 83(1): 209-216. https://doi.org/10.1253/circj.cj-18-0761
Samuchiwal SK, Balestrieri B, Raff H, Boyce JA (2017) Endogenous prostaglandin E2 amplifies IL-33 production by macrophages through an E prostanoid (EP)2/EP4-cAMP-EPAC-dependent pathway. Journal of Biological Chemistry 292(20): 8195-8206. https://doi.org/10.1074/jbc.m116.769422
Stillman BA, Audoly L, Breyer RM (1998) A conserved threonine in the second extracellular loop of the human EP2 and EP4 receptors is required for ligand binding. European Journal of Pharmacology 357(1): 73-82. https://doi.org/10.1016/s0014-2999(98)00522-6
Tsuge K, Iwasaki R, Morimoto K, Inazumi T, Kawahara O, Kawahara A, Tsuchiya S, Sugimoto Y (2013) Molecular and pharmacological characterization of zebrafish 'relaxant' prostanoid receptors. Biochemical and Biophysical Research Communications 436(4): 685-690. https://doi.org/10.1016/j.bbrc.2013.06.017
Wang P, Zhu F, Lee NH, Konstantopoulos K (2010) Prostaglandin E2 induces interleukin-6 expression in human chondrocytes via cAMP/protein kinase A- and phosphatidylinositol 3-kinase-dependent NF-κB activation. American Journal of Physiology-Cell Physiology 298(6): 1445-1456. https://doi.org/10.1152/ajpcell.00508.2009
Yokoyama U, Minamisawa S, Shioda A, Ishiwata R, Jin MH, Masuda M, Asou T, Sugimoto Y, Aoki H, Nakamura T, Ishikawa Y (2014) Prostaglandin E2 inhibits elastogenesis in the ductus arteriosus via EP4 signaling. Circulation 129(4): 487-496. https://doi.org/10.1161/circulationaha.113.004726
Zhu J, Mayr D, Kuhn C, Mahner S, Jeschke U, von Schonfeldt V (2018) Prostaglandin E2 receptor EP1 in healthy and diseased human endometrium. Histochemistry and Cell Biology 149(2): 153-160. https://doi.org/10.1007/s00418-017-1616-y