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Review

Evolution of endothelin receptors in vertebrates

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ABSTRACT

Endothelin receptors are G protein coupled receptors (GPCRs) of the β-group of rhodopsin receptors that bind to endothelin ligands, which are 21 amino acid long peptides derived from longer prepro-endothelin precursors. The most basal Ednr-like GPCR is found outside vertebrates in the cephalochordate amphioxus, but endothelin ligands are only present among vertebrates, including the lineages of jawless vertebrates (lampreys and hagfishes), cartilaginous vertebrates (sharks, rays, and chimaeras), and bony vertebrates (ray-finned fishes and lobe-finned vertebrates including tetrapods). A *bona fide* endothelin system is thus a vertebrate-specific innovation with important roles for regulating the cardiovascular system, renal and pulmonary processes, as well as for the development of the vertebrate-specific neural crest cell population and its derivatives. Expectedly, dysregulation of endothelin receptors and the endothelin system leads to a multitude of human diseases.

Despite the importance of different types of endothelin receptors for vertebrate development and physiology, current knowledge on endothelin ligand–receptor interactions, on the expression of endothelin receptors and their ligands, and on the functional roles of the endothelin system for embryonic development and in adult vertebrates is very much biased towards amniote vertebrates. Recent analyses from a variety of vertebrate lineages, however, have shown that the endothelin system in lineages such as teleost fish and lampreys is more diverse and is divergent from the mammalian endothelin system. This diversity is mainly based on differential evolution of numerous endothelin system components among vertebrate lineages generated by two rounds of whole genome duplication (three in teleosts) during vertebrate evolution.

Here we review current understanding of the evolutionary history of the endothelin receptor family in vertebrates supplemented with surveys on the endothelin receptor gene complement of newly available genome assemblies from phylogenetically informative taxa. Our assessment further highlights the diversity of the vertebrate endothelin system and calls for detailed functional and pharmacological analyses of the endothelin system beyond tetrapods.

1. The endothelin system and endothelin receptors

The endothelin system has a multitude of functions in vertebrates. Discovered in the late 1980s, it was first described to control blood pressure levels by vasoconstriction and vasodilation (Arai et al., 1990; Inoue et al., 1989; Masaki, 2004; Sakurai et al., 1990; Yanagisawa et al., 1988). Further on, it was found that the endothelin system functions also in many other aspects of vertebrate physiology and development, such as neurotransmission, wound healing, kidney homeostasis, osmoregulation (in fish), neural crest cell development, and many more (Barton and Yanagisawa, 2008; Hyndman and Evans, 2007; Khimji and Rockey, 2010; Rubanyi and Polokoff, 1994). Misregulation of the endothelin system leads to a multitude of pathological conditions such as cardiovascular, renal, pulmonary, and central nervous system diseases, atherosclerosis, ovarian cancer, and – due to its role in neural crest development – to problems with the development of the enteric nervous system, pigment cells, and the craniofacial skeleton (Bagnato and Rosano, 2008; Kedzierski and Yanagisawa, 2001; Khimji and Rockey, 2010; Pla and Larue, 2003; Schneider et al., 2007; von Websky et al., 2009).

At its core, the endothelin system consists of endothelin ligands, 21 amino acid long peptides processed from larger precursor prepro-endothelin proteins, that bind to G-protein coupled receptors

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(GPCRs) called endothelin receptors (Ednrs) (Fig. 1) belonging to β-group of rhodopsin receptors (Fredriksson et al., 2003). Vertebrate endothelin receptors contain seven transmembrane domains and are usually encoded by seven coding exons. A structurally annotated alignment of representative Ednr protein sequences is presented in Supplementary Fig. S1.

Upon Edn ligand binding, endothelin receptors can induce a variety of intracellular signaling cascades leading to diverse cellular responses such as contraction in the case of smooth muscle cells, or cell growth and mitogenesis. Ednrs are expressed in a variety of cell types and tissues, for example in endothelial and smooth muscle cells, cardiomyocytes of the heart, in the central and enteric nervous system, and in pigment cells, where they exert their multiple physiological and developmental roles (Khimji and Rockey, 2010; Rubanyi and Polokoff, 1994; Schneider et al., 2007).

The importance of endothelin signaling in multiple diseases has lead to the advancement of pharmacological strategies to interfere with the endothelin axis. A growing number of endothelin antagonists, that for example competitively inhibit the binding of endothelin ligands to the receptors, are available for the treatment of specific types of diseases and cancers (for reviews, see e.g., Bagnato et al., 2011; Battistini et al., 2006; Motte et al., 2006).

Almost 25,000 articles on the endothelin system have been published in the 25 years since its discovery, but studies that address the evolution and function of the endothelin system outside mammals are still comparatively scarce. While the human genome encodes three endothelin ligands (EDN1, EDN2, and EDN3) and two endothelin receptors (EDNRA and EDNRB), recent analyses from a variety of vertebrate lineages highlighted the diversity of the vertebrate endothelin system with up to seven ligand genes in lampreys (Kuraku et al., 2010) and five endothelin receptors in teleost fish (Braasch et al., 2009; Hyndman et al., 2009). Here we review recent advances in our understanding on the evolution of the endothelin GPCR gene family and their ligands, complemented by the analysis of *Ednr* gene repertoires in new genome assemblies from phylogenetically informative taxa such as amphioxus, lampreys, and cartilaginous, ray-finned, and lobe-finned fish.

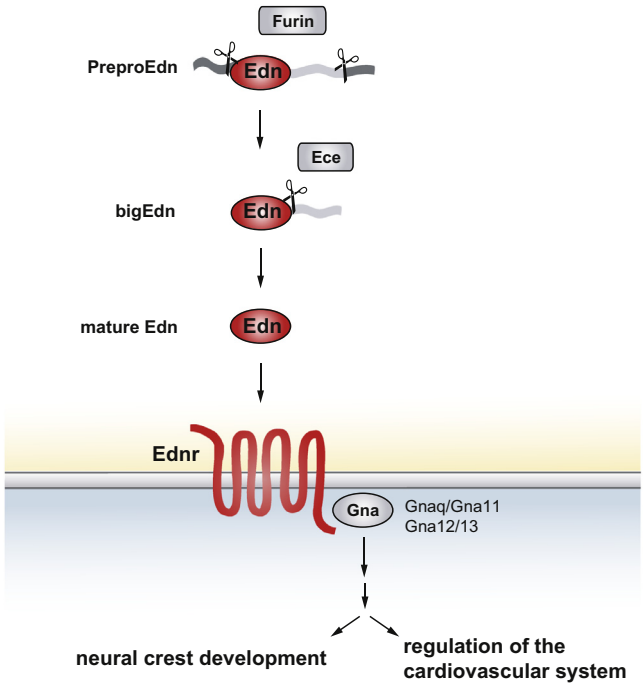

Fig. 1. The endothelin signaling system. The big endothelin (bigEdn) peptide is cleaved from preproendothelin (PreproEdn) protein by furin-like endopeptidases and further processed by endothelin-converting enzymes (Ece) into the mature endothelin ligand peptide (Edn). Endothelins bind to the G protein-coupled endothelin receptors (Ednr) and by signaling through a variety of G proteins regulate the cardiovascular system, neural crest development and many other processes (reviewed in Barton and Yanagisawa, 2008; Khimji and Rockey, 2010).

2. Evolutionary origins of the endothelin system

2.1. Emergence of the endothelin system at the base of vertebrates

When did the endothelin receptors and the endothelin system as a whole emerge during the course of eukaryote evolution? Surprisingly, endothelin peptides can induce chemotactic behavior in the unicellular ciliate *Tetrahymena* (Kohidai et al., 2001) and muscle contractions in the cnidarian *Hydra* (Zhang et al., 2001), and endothelin-like immunoreactivity has been observed for mollusks, insects, and the urochordate *Ciona intestinalis* (Kasuya et al., 1991). These observations suggested that the endothelin system might be in place in these lineages and thus evolutionarily very old. Indeed, the enzymatic machinery that in vertebrates cleaves the preproendothelin peptides into the mature endothelin ligand, i.e. furin and endothelin-coverting enzymes (Ece) (Fig. 1), are found in all major animal lineages including cnidarians and even in fungi, bacteria, and archea (Bland et al., 2008; Day and Strongin, 2011; Hyndman and Evans, 2007; Macours et al., 2004; Zhang et al., 2001).

Genes encoding endothelin peptides, however, have never been found outside vertebrates and are thus considered a vertebrate novelty (Braasch et al., 2009; Hyndman and Evans, 2007; Jekely, 2013; Kuraku, 2012; Martinez-Morales et al., 2007). Furthermore, endothelin receptor genes have so far only been identified in chordates (Braasch et al., 2009; Jekely, 2013; Mirabeau and Joly, 2013). In additional analyses making use of recently available genomic information, we failed to find *Ednr* genes in the genomes of non-chordate deuterostomes, i.e., the sea urchin *Strongylocentrotus purpuratus* and the acorn worm *Saccoglossus kowalevskii*, in protostomes, in the cnidarians *Nematostella vectensis* and *Hydra magnipapillata*, in the placozoan *Trichoplax adhaerens*, or in the sponge *Amphimedon queenslandica*. It therefore appears that the class of endothelin GPCRs is indeed chordate-specific (Braasch et al., 2009; Jekely, 2013; Mirabeau and Joly, 2013).

Among chordates, Ednrs have been found in all vertebrate lineages (Braasch et al., 2009; Kuraku et al., 2010) and a hypothetical Ednr-like protein is described for the cephalochordate *Branchiostoma floridae* (BRAFLDRAFT_155652) (Braasch et al., 2009; Nordstrom et al., 2008) representing the most basally diverging chordate lineage. In contrast, no *Ednr* gene is present in the urochordate genomes of *C. intestinalis* and *Oikopleura dioca*. As tunicates are more closely related to vertebrates than cephalochordates (Delsuc et al., 2006) this suggests that an *Ednr*-like gene was lost secondarily in urochordates (Braasch et al., 2009).

2.2. Orthology of cephalochordate Ednr-like and vertebrate Ednr genes

The closest related GPCR genes to the *Ednr* genes in vertebrates are the *G-protein coupled receptor 37* (*Gpr37*) and *G protein-coupled receptor 37 like 1* (*Gpr37l1*) genes and, more distantly, the group of *Gastrin-releasing peptide receptor* (*Grpr*), *Neuromedin B receptor* (*Nmbr*), and *Bombesin-like receptor 3* (*Bsr3*) genes (Braasch et al., 2009; Fredriksson et al., 2003; Hyndman et al., 2009; Nordstrom et al., 2008). The fact that amphioxus possesses orthologs for each of these vertebrate genes (Braasch et al., 2009; Nordstrom et al., 2008) makes the possibility that BRAFLDRAFT_155652 is an amphioxus ortholog of any of these genes unlikely.

Using the Synteny Database (Catchen et al., 2009), we are able to establish for the first time conserved synteny between the