BJP British Journal of Pharmacology

DOI:10.1111/bph.12718
www.brjpharmacol.org

COMMENTARY

EPH receptor/ephrin system: in the quest for novel anti-angiogenic therapies: Commentary on Hassan-Mohamed *et al.*, Br J Pharmacol 171: 5195–5208

M Hatziapostolou and C Polytarchou

Center for Systems Biomedicine, Division of Digestive Diseases, David Geffen School of Medicine, University of California Los Angeles, Los Angeles, CA, USA

Correspondence
Christos Polytarchou, Center for Systems Biomedicine, Division of Digestive Diseases, David Geffen School of Medicine, University of California Los Angeles, Los Angeles, CA 90095, USA. E-mail: cpolytarchou@mednet.ucla.edu

Keywords
EPH receptors; ephrins; angiogenesis inhibitors; cancer

Received
26 March 2014
Accepted
4 April 2014

LINKED ARTICLE
This article is a Commentary on Hassan-Mohamed I, Giorgio C, Incerti M, Russo S, Pala D, Pasquale EB, Zanotti I, Vicini P, Barocelli E, Rivara S, Mor M, Lodola A and Tognolini M (2014). UniPR129 is a competitive small molecule Eph-ephrin antagonist blocking in vitro angiogenesis at low micromolar concentrations. Br J Pharmacol 171: 5195–5208. doi: 10.1111/bph.12669

Abbreviations
Eph, EPH (ephrin) receptor; Trp, tryptophan; VEGFR, VEGF receptor

This is a Commentary on an article in BJP by Hassan-Mohamed *et al.*, 2014; 171: 5195–5208. The term angiogenesis is generally applied to the growth of microvessel sprouts the size of capillary blood vessels, a process that is orchestrated by a range of angiogenic factors and inhibitors. From embryonic development to adulthood, blood vessels play a fundamental physiological role in supplying oxygen and nutrients, removing catabolic waste, and circulating cells for immune surveillance. However, aberrant angiogenesis occurs in a range of diseases that could be classed together as 'angiogenesis-dependent diseases'. Angiogenesis is an early- to midstage event in many human cancers and is crucial for tumours to grow beyond a microscopic size and metastasize to distant sites throughout the body (Folkman, 2007).

In 1971 Judah Folkman hypothesized that starving a tumour of its blood supply may be therapeutic. Since then, much effort has focused on identifying new therapeutic agents that inhibit pathological angiogenesis. Most of the anti-angiogenic agents that have entered the drug development pipeline in the past decade target the main pro-angiogenic VEGF signalling pathway, at the level of the ligand and its receptors (VEGFR) (Moreno Garcia *et al.*, 2012). Antibody-mediated inhibition of VEGF using bevacizumab is currently the predominant mode of VEGF-targeted therapy, although drugs that inhibit VEGF receptor tyrosine kinase activity (such as sunitinib and sorafenib) are also used (Goel and Mercurio, 2013). However, in both preclinical and clinical settings, resistance mechanisms have limited the long-term benefit of VEGF-targeted strategies. The main mechanisms of the resistance to VEGF signalling blockade include signalling by redundant receptors, such as the fibroblast growth factors, angiopoietin-1 and ephrins. Hence, currently research has mainly focused on how to improve existing anti-angiogenic therapies, and data from preclinical

studies suggest that combined inhibition of different pro-angiogenic factors could be a suitable strategy.

EPH receptors (Eph), the largest family of receptor tyrosine kinases (see Alexander *et al*., 2013), and their cell-bound ephrin ligands represent an essential cell communication system with widespread effects on the actin cytoskeleton, cell shape and motility, cell–cell and cell–matrix contacts in a number of biological processes. In addition, effects on cell proliferation, survival, differentiation and secretion have also been demonstrated. The 16 EPH receptors and 9 ephrins are divided into two subclasses A or B based on binding properties and structural homologies. Although class A receptors preferentially bind A-type ligands and the class B receptors bind B-type ligands, some interclass binding examples exist. Because both receptors and ligands are membrane-bound, their interactions at sites of cell–cell contact initiate unique bidirectional signalling cascades that affect both receptor- and ephrin-expressing cells. The signalling downstream of the EPH receptor is referred to as 'forward' and the signalling downstream of the ephrins is referred to as 'reverse'. Although bidirectional signalling is their best characterized modus operandi, EPH receptors and ephrins may also function independently of each other and/or in concert with other cell-surface communication systems such as receptors, adhesion molecules, channels and cell-surface proteases (Mosch *et al*., 2010; Boyd *et al*., 2014).

The Eph/ephrin system is widely expressed in embryogenesis and has a leading role in the developmental tissue patterning, in particular in the CNS and CVS. Additionally, it is a crucial regulator of many physiological activities in the adult, including the plasticity and regenerative capacity of the nervous system, angiogenesis, glucose homeostasis, immunological and inflammatory host responses, intestinal homeostasis, bone morphogenesis and stem cell plasticity. Eph/ephrin signalling not only has a role in physiological, but also in pathophysiological processes; an imbalance in the Eph/ephrin system has been reported in a variety of pathologies, such as cancer, diabetes and Alzheimer's disease (Pasquale, 2008).

Currently, the most intensely studied function of the Eph/ephrin system is that during development and progression of different cancers. The expression of many ephrins and EPH receptors is aberrant in several human carcinomas and their functions have been implicated in tumour progression, angiogenesis and metastasis of a large range of epithelial and mesenchymal carcinomas. The mechanisms that control the expression and function of Eph/ephrins are somatic mutations, epigenetic silencing and mRNA stability. The up-regulation and/or down-regulation of Eph/ephrin proteins in many human cancers highlight the complexity of the Eph/ephrin system in tumour progression and metastasis. EPH receptors do not behave like classical oncogenic growth factor receptors and the dichotomy of the ephrin system between oncogenic and anti-angiogenic properties have been well described for EPH receptors B2 and B4 (EphB2 and EphB4). Overexpression of these EPH receptors in colon, breast and endometrial cancers correlates with tumour cell survival, disease progression and poor disease outcomes. In contrast, other studies have suggested that activated EphB4 triggers breast cancer cell apoptosis and is tumour suppressive in colon cancer (Noren and Pasquale, 2007; Boyd *et al*., 2014).

Besides being expressed in cancer cells, EPH receptors and ephrins are also present in the tumour vasculature, where they promote angiogenesis, an important facet of cancer. Based on a series of *in vitro* and *in vivo* experiments with mouse tumour models, the main roles in tumour angiogenesis have so far been attributed to EphA2 forward signalling and ephrin-B2 reverse signalling. Interestingly, EphA2 appears to be required for VEGF-induced endothelial cell migration and assembly into capillary-like structures (Chen *et al*., 2006). The prominent role of ephrin-B2 and its receptor EphB4 in tumour-derived angiogenesis and tumour growth has also been well documented. In particular, ephrin-B2 reverse signalling regulates endothelial tip cell guidance through the regulation of VEGFR2 internalization, a key event in the VEGFR activation and downstream signalling pathway. Impaired signalling decreases tumour vascularization and growth suggesting that ephrin-B2-mediated reverse signalling inhibition might be an alternative or combinatorial anti-angiogenic therapy to disrupt VEGFR2 function in tumour angiogenesis (Sawamiphak *et al*., 2010).

Numerous strategies have been employed to target the Eph–ephrin system and several are currently under preclinical or clinical testing: monoclonal antibodies functioning either as agonists or antagonists of specific EPH receptors, fusion proteins interfering with the Eph–ephrin interaction, small molecules blocking the EPH receptor downstream signalling and small interfering RNAs for inhibiting the expression of EPH receptors. Antibody-based therapeutics targeting EPH receptors found to be overexpressed in specific cancers, show anti-angiogenic as well as antitumour effects. Despite the fact that the first clinical trial targeting EphA2 has been terminated because of drug toxicity, results from preliminary studies support the targeting of EphA3 as a therapy for haematological cancers. Fusion proteins functioning as agonists utilize the suppressive role of EphB2 and EphB4 in colon, breast and prostate tumours, while the oncogenic role of EphA2 has lead to the development of proteinic antagonists. Several kinase inhibitors aimed primarily at other targets have been shown to target the activity of EPH receptors and are currently undergoing clinical or preclinical evaluations as standalone drugs or in combination with chemotherapy. However, the limited efficacy and specificity of ATP-competitive agents may represent important obstacles in their clinical applications (Boyd *et al*., 2014).

Alternatively, a group of small molecules that inhibit the interaction between ephrin and the EPH receptor is currently under development. These molecules have several advantages, in that they may inhibit the bidirectional signals, their action does not depend on cell membrane permeability and can be designed for increased specificity. Among them, lithocholic acid, a secondary bile acid, has been used as the basis for the development of small molecules targeting the ephrin system. In a recent paper in *British Journal of Pharmacology*, Hassan-Mohamed *et al*. (2014) report on a novel protein–protein inhibitor that disrupts the interaction between EphA2 and ephrin-A1 at low micromolar concentrations. Using a computational approach, based on a tryptophan (Trp)-conjugate of lithocholic acid, the authors investigated for molecules with higher affinity for EphA2. Thus, they designed and synthesized an Eph–ephrin inhibitor (UniPR129) predicted to interact more efficiently with the

ligand-binding pocket of the receptor. Indeed, pharmacological and biochemical analyses verified the ability of a modified Trp on UniPR129 to potentiate this interaction. In the same line, *in vitro* studies revealed that this compound inhibited the activation of EphA2 in cells. Importantly, at low micromolar non-cytotoxic concentrations, UniPR129 inhibited the ability of endothelial cells to migrate and form capillary-like tubes *in vitro*, two features required for angiogenesis *in vivo*. While its *in vivo* efficacy remains to be determined, its increased affinity for EPH receptors in combination with the lack of cytotoxic effects renders this new lithocholic acid derivative a candidate anti-angiogenic agent (Hassan-Mohamed *et al.*, 2014).

With our current understanding of the causative role of specific ephrins and EPH receptor family members in different diseases, the question remains as to the specificity and efficacy of therapeutic targeting. Given the involvement of the Eph–ephrin system in the function of various organs, restricting the effects of a potential inhibitor in the diseased tissue or limiting its toxicity are emerging tasks.

Conflict of interest

None.

References

Alexander SPH, Benson HE, Faccenda E, Pawson AJ, Sharman JL, Spedding M, Peters JA, Harmar AJ and CGTP Collaborators (2013). The Concise Guide to PHARMACOLOGY 2013/14: Catalytic receptors. Br J Pharmacol 170: 1696–1705.

Boyd AW, Bartlett PF, Lackmann M (2014). Therapeutic targeting of EPH receptors and their ligands. Nat Rev Drug Discov 13: 39–62.

Chen J, Hicks D, Brantley-Sieders D, Cheng N, McCollum GW, Qi-Werdich X *et al.* (2006). Inhibition of retinal neovascularization by soluble EphA2 receptor. Exp Eye Res 82: 664–673.

Folkman J (2007). Angiogenesis: an organizing principle for drug discovery? Nat Rev Drug Discov 6: 273–286.

Goel HL, Mercurio AM (2013). VEGF targets the tumour cell. Nat Rev Cancer 13: 871–882.

Hassan-Mohamed I, Giorgio C, Incerti M, Russo S, Pala D, Pasquale EB *et al.* (2014). UniPR129 is a competitive small molecule Eph–ephrin antagonist blocking *in vitro* angiogenesis at low micromolar concentrations. Br J Pharmacol 171: 5195–5208.

Moreno Garcia V, Basu B, Molife LR, Kaye SB (2012). Combining antiangiogenics to overcome resistance: rationale and clinical experience. Clin Cancer Res 18: 3750–3761.

Mosch B, Reissenweber B, Neuber C, Pietzsch J (2010). Eph receptors and ephrin ligands: important players in angiogenesis and tumor angiogenesis. J Oncol 2010: 135285.

Noren NK, Pasquale EB (2007). Paradoxes of the EphB4 receptor in cancer. Cancer Res 67: 3994–3997.

Pasquale EB (2008). Eph–ephrin bidirectional signaling in physiology and disease. Cell 133: 38–52.

Sawamiphak S, Seidel S, Essmann CL, Wilkinson GA, Pitulescu ME, Acker T *et al.* (2010). Ephrin-B2 regulates VEGFR2 function in developmental and tumour angiogenesis. Nature 465: 487–491.