

A REVIEW OF PROTECTIVE EFFECT OF EPHRIN RECEPTOR AGONIST DOXAZOSIN IN MYOCARDIAL INJURY

Komal^{1*}, Kapil Kumar Verma¹, Panshul Sharma¹

¹Minerva College of Pharmacy, Indora, Kangra-176402

ABSTRACT

The biggest family of receptor tyrosine kinases, eph receptors interact with membrane-bound ephrin ligands to regulate a wide range of vital human activities, from embryonic development to adult tissue homeostasis. Eph receptors and ephrin ligands are widely expressed throughout immune system and cardiovascular system, which emphasises how crucial these molecules are for coordinating the best response. The numerous functions of Eph receptors and ephrin ligands. In their interaction with Eph receptors might be a potential therapeutic target in treatment of myocardial infarction are summarised in this study. We also go over the significance of Ephrin-Eph signalling has been shown to reduce inflammation and apoptosis after an ischemic insult and promote angiogenesis. Doxazosin has been reported to activate EphA2 receptors at 25 µM and 50µM. in HEK and PC-3 cells respectively. Oxidative stress generated within first few minutes of myocardial reperfusion has been reported in myocardial injury and cardiomyocyte death through a number of differ mechanisms that PI3/Akt pathway may be involved in cardio protective effect of Doxazosin downstream Ephrin A2 receptor activation. The numerous and important functions investigated of Eph receptors and ephrin ligands throughout the system both when it is dormant and active.

Keywords: Myocardial infarction, Eph receptors, Oxidative stress, Doxazosin, Angiogenesis.

INTRODUCTION

Acute myocardial infarction is a disease which affects the patient in an extremely stressful way [1]. Myocardial infarction (MI) is also known as heart attack. It is a condition of heart muscle death when one or more coronary arteries which supply oxygen-rich blood to the heart muscle become suddenly blocked due to thrombosis superimposed over previously atherosclerotic coronary artery [2]. This leads to a sudden obstruction of blood flow to myocardial tissue resulting in infarction. The longer the duration of ischemia the greater the amount of heart muscle dies.

Timely reperfusion is necessary for salvage of ischemic myocardium from irreversible damage [3]. However reperfusion itself independently result in cardiomyocyte dysfunction and death, termed as 'Reperfusion Injury' [4]. Novel therapeutic targets that would increase the survival of cardiomyocyte or prevent apoptosis in response to myocardial ischemia reperfusion injury are under investigation. Ephrin (erythropoietin-producing hepatocellular carcinoma) is a large family of receptor tyrosine kinases with important roles in tissue organization and growth during development as well as in adult tissue homeostasis [5-7]. Importantly, Eph receptors together with their ligands, the ephrins (Eph receptor interacting proteins), represent key players in many pathological conditions and therefore promising drug targets [8-10].

Eph-A receptors and ephrin-A1 ligand are expressed in healthy myocardium and expression increased post myocardial infarction [11,12]. Thus, Ephrin ligands and their interaction with the Eph receptors might be a potential therapeutic target in treatment of MI [11-13]. Intramyocardial administration of EphrinA1-Fc has been reported to increase expression of Eph A1, Eph A2 receptors, Eph-A4 protein and improve cardiomyocyte survival through increased phosphorylated Akt protein expression [11]. EphA4 receptor activation leads to anti-apoptotic effect, while inhibition or removal of the receptor is pro-apoptotic. Mice lacking EphA4 have thymic hypoplasia and decreased peripheral T-cells due to increased apoptosis and reduced cell cycling [14].

Ephrin-Eph signalling has been reported to modulate myocardial repair or regeneration after MI. The ephrinB2 chimeric protein, ephrinB2-Fc, also induced endothelial cell proliferation and sprouting when used *in vitro*. Following MI, mice that were treated with an injection of ephrinB2-Fc had a 28% increase in capillary density in the peri-infarct region [15]. EphrinA1-Fc would accelerate revascularization and thereby improve myocardial infarct healing [16].

Doxazosin, a Piperazinyl quinazoline compound, is a long lasting selective inhibitor of α1-adrenergic receptors. It is a commonly prescribed treatment for patients with hypertension^{17,18} and enlarged prostate [19].

Doxazosin has been reported as a novel small molecule agonist of EphA2 and EphA4 receptors in Human embryonic kidney 293 cell lines. Ephrin-Eph signalling has been shown to reduce inflammation and apoptosis after an ischemic insult and promote angiogenesis.

EPHRIN RECEPTORS

The largest known family of receptor tyrosine kinases in mammals, the Eph (erythropoietin-producing hepatocellular carcinoma) receptors, are found in such cells [20]. These receptors serve as important mediators of adult tissue homeostasis and are crucial for a variety of typical cellular processes during development [21-24]. First found in a cell line from a human cancer [25]. The Eph receptors exhibit the classic RTK topology, which acts as a single transmembrane segment, a multidomain extracellular region with the ephrin ligand-binding domain, and a cytoplasmic region with the kinase domain (Fig. 1). The human genome contains five EphB receptors that promiscuously bind three EphrinB ligands and nine EphA receptors that promiscuously bind five ephrin-A ligands [26]. Ephrins of a different class can also be bound by EphA4 and EphB2. The kinase domains of two

members of the family, EphA10 and EphB6, contain changes that stop kinase activity. Eph receptors have unique activities and a different structure from the prototypical form [27].

EFFECTS OF EPHRIN RECEPTORS ON MYOCARDIAL DISEASE

Myocardial Infarction

Myocardial infarction is a clinical syndrome of coronary artery disease and is one of the leading cause of death and disability worldwide [29]. The complete blockage of a coronary artery caused by a rupture of an atherosclerotic plaque is usually the underlying mechanism of Myocardial infarction (MI). This leads to insufficient blood supply which results in inflammation (necrosis) of myocardial tissue, ventricular remodelling and left ventricular dysfunction [30].

The prognosis after myocardial infarction varies greatly depending on the extent and location of the affected heart muscle, and the development and management of complications. Prognosis is worse with older age, and social isolation. Anterior infarcts, persistent ventricular tachycardia or fibrillation, development of heart blocks, and left ventricular impairment are all associated with poorer prognosis [31].

MYOCARDIAL ISCHEMIA – REPERFUSION INJURY

Early reperfusion is necessary to salvage ischemic myocardium. Successful myocardial reperfusion with thrombolytic agents, primary percutaneous coronary intervention and coronary artery bypass grafting (CABG) is most effective strategy to reduce the infarct size. However, the process of myocardial reperfusion can itself induce further cardiomyocyte death, a phenomenon known as “Myocardial Ischemia-Reperfusion Injury”^[32]. Reperfusion injury is manifested as:

- (i) Reperfusion arrhythmias.
- (ii) Microvasculature damage;
- (iii) Stunned myocardium, where myocytes display a prolonged period of contractile dysfunction following coronary blood flow restoration; and
- (iv) ‘Irreversible IR injury’- cardiomyocyte death due to prolonged ischemia followed by reperfusion.

Ischemia causes decreased oxidative phosphorylation due to a lack of oxygen in the mitochondrial respiratory chain. Adenosine triphosphate (ATP) synthesis is shifted to the glycolytic pathway which leads to an intracellular accumulation of lactic acid [4]. This causes intracellular pH to rapidly fall to almost 6.4 within minutes [33,34]. Acidosis and accumulation of inorganic phosphate rapidly halt contractile activity. The ATP concentration continues to fall, reaching critical [35]. Acidosis activates sarcolemmal Na^+/H^+ exchangers, with a consequent influx of Na^+ , while the energy depletion stops Na^+ efflux through Na^+/K^+ ATPase. Consequently, Na^+ accumulates in the interior of the cell, activating $\text{Na}^+/\text{Ca}^{2+}$ exchangers in the reverse direction and increasing cytosolic Ca^{2+} . Restoration of myocardial blood flow leads to a recovery in respiratory activity, mitochondrial membrane potential, and ATP synthesis. The cell membrane potential is restored, which, in the presence of a high cytosolic concentration of Na^+ , causes the $\text{Na}^+/\text{Ca}^{2+}$ exchanger to function in reverse, further exacerbating the cytosolic Ca^{2+} overload. High cytosolic Ca^{2+} levels can have profound negative effects on the cardiomyocyte during reperfusion inducing hypercontracture, electrical instability, and contractile dysfunction. Ca^{2+} overload is a major activator of the mitochondrial permeability transition pore (mPTP) in reperfusion, a response associated with reactive oxygen species (ROS) generation and the initiation of pro-death pathways [37].

Thus, calcium overload, pH recovery and ROS overproduction are major players in determining Ischemia Reperfusion injury [38].

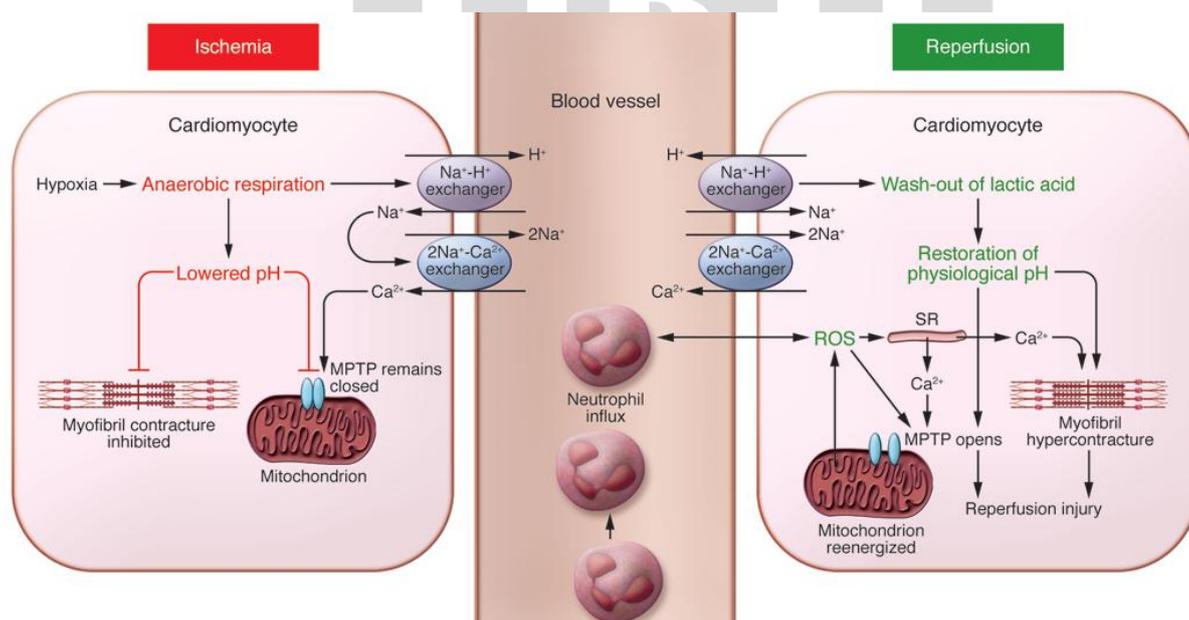


Figure 1: Main components of Myocardial Ischemia Reperfusion Injury

During acute myocardial ischemia, the absence of oxygen switches cell metabolism to anaerobic respiration, resulting in the production of lactate and a drop in intracellular pH. This induces the Na^+/H^+ exchanger and results in intracellular Na^+ overload,

which activates the $2\text{Na}^+ - \text{Ca}^{2+}$ exchanger to function in reverse to extrude Na^+ and leads to intracellular Ca^{2+} overload. During reperfusion, the electron transport chain is reactivated, generating ROS. Other sources of ROS include xanthine oxidase (endothelial cells) and NADPH oxidase (neutrophils). ROS mediate myocardial reperfusion injury by inducing the opening of the MPTP, acting as a neutrophil chemoattractant, and mediating dysfunction of the sarcoplasmic reticulum (SR). This contributes to intracellular Ca^{2+} overload and damages the cell membrane. Reperfusion and reactivation of the $\text{Na}^+ - \text{H}^+$ exchanger result in washout of lactic acid, resulting in the rapid restoration of physiological pH, which releases the inhibitory effect on MPTP opening and cardiomyocyte contracture. The restoration of the mitochondrial membrane potential drives calcium into the mitochondria, which can also induce MPTP opening [32].

EPHRIN RECEPTORS AND SIGNALING

The Ephrin (*Eph*) receptors represent the largest family of receptor tyrosine kinases [16] and were identified in the late 1980's. Eph receptors and their ephrin ligands are divided into the two subclasses A and B, based on the sequence homology of the extracellular sequence, structure and the binding affinity. The Eph receptor family, which includes 14 members, Eph receptors and their Ephrin (Eph receptor interacting) ligands form a system of cell communication with widespread roles in physiology and disease. In mammals, there are nine EphA (EphA1–8, and EphA10) receptors, which bind five glycosylphosphatidylinositol (GPI)-linked EphrinA ligands and five EphB (EphB1–4 and EphB6) receptors, which bind three transmembrane Ephrin B ligands⁴¹. There are some exceptions: EphA4 can bind both A-type and most B-type Ephrins, EphB2 can bind EphrinA5, and EphB4 essentially binds only EphrinB2. One of the unique features of Eph/ephrin signaling is the fact that both receptors and ligands are competent to transduce a signaling cascade upon interaction. Typically, Eph receptor–Ephrin ligand interactions occur at the cell's surface at sites of cell-to-cell contact and result in bidirectional signaling from the Eph receptor (forward signalling) and from the Ephrin ligand (reverse signaling) [41-44].

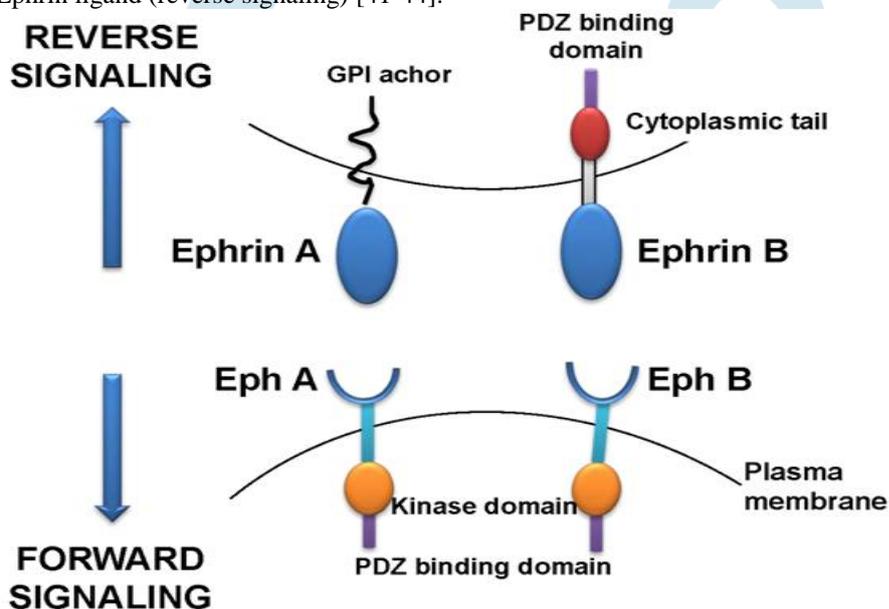


Figure 2: The Eph-ephrin Signaling

Structure of Ephrin Receptor

Ephrin receptors consist of a glycosylated extracellular domain with ligand-binding site, followed by a cysteine-rich region and two fibronectin type III repeats (Figure 3) Connected via a single transmembrane spanning domain, the intracellular region contains a juxtamembrane domain, a tyrosine kinase domain, a sterile alpha motif, and a PDZ-(Postsynaptic density 95-Discs large-Zonula occludentes-1) binding motif. Eph receptors bind membrane bound ligands, the ephrins, and both, receptors and ligands, are divided into two subclasses A or B based on binding properties and structural homologies. Class A ephrin are membrane-bound via a glycosylphosphatidylinositol anchor and class B ephrins contain a transmembrane domain and a short cytoplasmic region with conserved tyrosine residues and a PDZ-binding motif. Class A Eph receptors preferentially bind all A-type ephrins and class B Eph receptors bind all B-type ligands. However, there are some exceptions, as EphA1 primarily binds ephrinA1, EphA4 binds both, A and B-type ligands, and ephrinA5 binds EphA receptors as well as EphB2 [43].

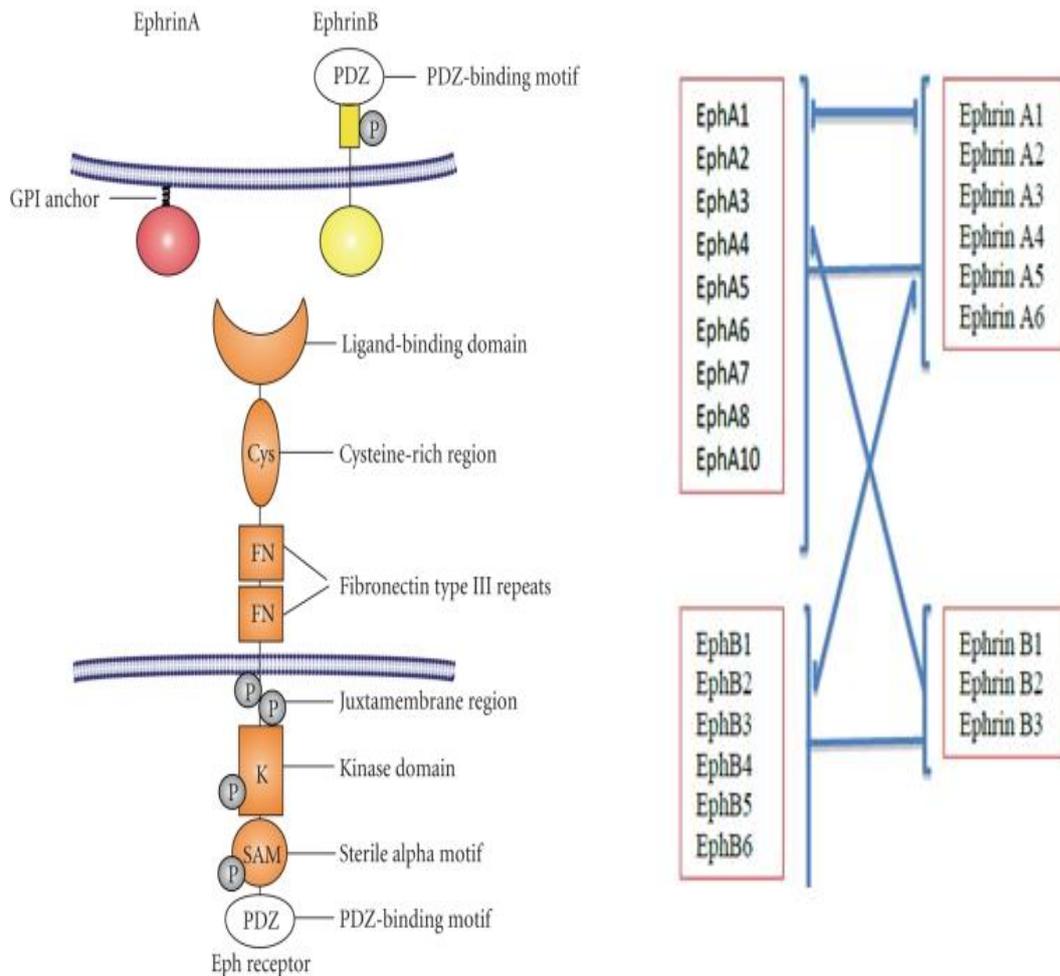


Figure 3: The structure of the Eph receptors and their ephrin ligands

PHYSIOLOGICAL ROLES OF EPHRIN RECEPTORS

Ephrin receptors have diverse activities, including widespread effects on the actin cytoskeleton, cell-substrate adhesion, intercellular junctions, cell shape, and cell movement [44,5]. In addition, effects on cell proliferation, survival, differentiation, and secretion have also been described. These activities depend on the interaction of the Eph receptors with the Ephrins (*Eph* receptor interacting proteins).

Neuron development and regeneration are one of process, in which Eph and ephrins receptors are involved. Ephrin system participates in communication between neurons as well as glial cells. Ephrins are present in an adult brain during remodeling processes due to external factors (in hippocampus, where quantity and size of the synapses are changing during processes corresponding to learning), whereas mutations connected with ephrins cause disorders such as Alzheimer disease and anxiety. Drugs antagonizing Eph/ephrin system may be a cure in these diseases.

Ephrins also play a role in the immunological process such as development of thymocytes into lymphocytes T in thymus [44]. Eph A is involved in glucose homeostasis. Forward signaling inhibits insulin secretion whereas reverse signaling acts opposite-increase insulin secretion. Homeostasis in adult bones depends on osteoblasts and osteoclasts activity. EphB/ephrin-B system during development due to gene mutation causes cleft palate, skull Vault and craniosynostosis. Dysfunction of this system may lead to disturbed bone homeostasis and osteoporosis .

Stem cells are capable of dividing and migration along the crypts. EphB/ephrin-B takes part in the differentiation and motility of cells by using intercellular repulsion. Overexpression of EphB 1, 2, 3 and 4 is present in cells at the bottom of the intestinal crypts, whereas EphB6 expression is dominating in surface cells [45].

EPHRIN RECEPTORS AND LIGANDS IN CARDIOVASCULAR SYSTEM

EphA2/ephrin-A1 and EphB4/ephrin-B2 play key roles in the adult cardiovascular system. The Eph/ephrin system is critical for cardiovascular development, as shown by the heart and blood vessel defects resulting from knockout of Eph receptors or ephrins. EphA3 and ephrin-A1 are expressed in adjacent cells of the developing mouse heart, and that EphA3 mice develop debilitating endocardial cushion and atrial septal defects that lead to death in most mutants. Ephrin-A1 is expressed in adult healthy myocardium and blood vessels [12] and is also involved in vascular development. In EphA2-deficient mice abnormalities in capillary development has been revealed.

EphrinB2 and its receptor EphB4 play critical roles in the development of the vascular

system and contribute to the function of the adult vasculature. Knockout mice lacking EphrinB2 or EphB4 expression and mice with deletion of Ephrin B2 targeted to the endothelial cells display a severely compromised vascular system and die at mid gestation. The targeted deletion of EphrinB2 in mural cells (pericytes and smooth muscle cells) leads to diffuse tissue edema, haemorrhaging, and perinatal death of the mice. Further, EphB6 and ephrin-B1 expressed in vascular smooth muscle cells can contribute to blood pressure regulation. Ephrin-Eph signalling has also been reported to be pro-angiogenic. The Eph/ephrin system controls blood vessel sprouting, assembly, remodelling and stabilization by regulating endothelial cells and their supporting mural cells, including pericytes and vascular smooth muscle cells. EphA2/ephrin-A1 and EphB4/ephrin-B2 plays a key role in regenerative and pathological forms of angiogenesis. EphA2 expression in angiogenic vasculature but low to undetectable in embryonic and postnatal quiescent vasculature.

EphA2 regulates angiogenesis and vascular permeability mainly in concert with ephrin-A1 also expressed in endothelial cells, through a mechanism that involves interplay with vascular endothelial growth factor (VEGF). With regard to the EphB/ephrin-B system, EphB4 is preferentially expressed in venous endothelial cells and its ligand ephrin-B2 in arterial endothelial cells, and both are involved in arterial-venous specification. Ephrin-B2 is tyrosine phosphorylated in angiogenic but not in quiescent vasculature, consistent with an active signaling function linked to blood vessel sprouting and remodeling. Inhibition of phosphorylation dependent or PDZ- dependent signalling down-stream of EphrinB ligands prevents endothelial cell sprouting⁴⁷. Ephrin-B2 and EphB4 are also involved in pathological forms of postnatal angiogenesis, such as neovascular disorders of the eye. Interestingly, ephrin-B2 is upregulated by VEGF and is in turn required for VEGF receptor endocytosis and angiogenic signaling.

EPHRIN RECEPTORS AS THERAPEUTIC TARGETS IN MYOCARDIAL INFARCTION AND ISCHEMIA REPERFUSION INJURY

Several studies have investigated the role of ephrin-Eph signalling in the context of myocardial repair or regeneration after MI. It is reasonable to suggest that these cell surface proteins may promote angiogenesis or reduce Cardiomyocyte death after an ischemic insult.

Ephrin signalling mediated angiogenesis and infarct healing.

Angiogenesis represents the newly formed microvessels from pre-existing capillaries. [48]. Angiogenesis is essential for recovery after tissue damage. It occurs in healing wounds, and is important in recovery after cardiac and skeletal muscle ischemia [49]. Angiogenesis has the potential to salvage ischemic myocardium at early stages after MI by promoting infarct healing. During infarct healing or remodelling, angiogenesis occurs in the granulation tissue that will ultimately form the infarct scar, and neovascularization occurs in surrounding, viable myocardium in the infarct border zone [50].

Eph B2 is suggested to be of importance in postnatal and adult angiogenesis. Ephrin B2 has a stimulatory effect on the migration of microvascular endothelial cells *in vitro* and hypoxia is reported to upregulate the expression of ephrin B2 in the mouse skin. Hypoxia inducible factor- (HIF-1 α) has been reported to involve in mediating hypoxia-dependent up-regulation of Eph receptors/ephrins expression in cultured Hep3B and PC-3 cells. Myocardial ischemia results in tissue hypoxia, that leads to induction of Hypoxia-inducible transcription factors, such as HIF1- α that mediate cellular adaptation to hypoxia and control both developmental and postnatal angiogenesis [51]. HIF1- α is activated by hypoxia and many proangiogenic genes. HIF1- α and HIF2- α proteins are expressed in cardiomyocytes, endothelial, and inflammatory cells early after MI [52]. Mice constitutively expressing HIF1- α in cardiomyocytes display improved cardiac function after MI, associated with increased VEGF expression and angiogenesis in the myocardium [53]. HIF-1 α controls the extent of endothelial progenitor cell (EPC) recruitment to ischemic areas and angiogenesis. HIF-2 α is also able to induce expression of various angiogenic genes, such as VEGF or angiopoietins recently been shown to be essential for ischemia induced angiogenesis [53]. Further, in a model of hind limb ischemia in mice, ephrin B2 mRNA was after 24 hr upregulated in the ischemic area [54].

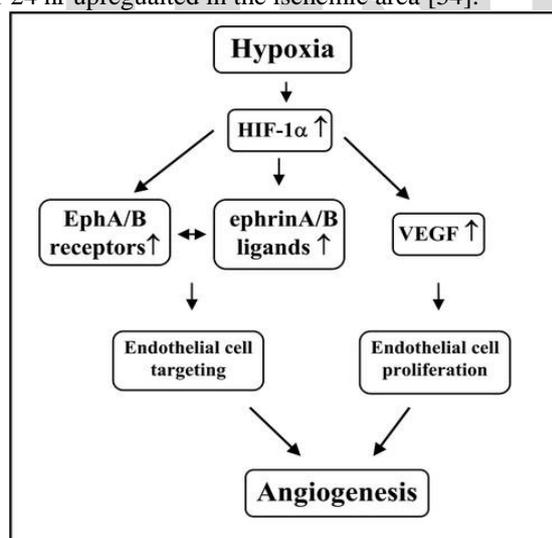


Figure 4: Role of HIF - 1 α and ephrin receptor in angiogenesis

Ephrin B2 also directly controls VEGF-induced angiogenesis. Ephrin A1 is induced by VEGF in HUVEC cells and microvascular endothelial cells. Blocking EphA receptor signalling inhibit VEGF-induced endothelial cell survival, migration, sprouting *in vitro* and corneal angiogenesis *in vivo*, suggesting EphA receptor activation is required for VEGF-induced angiogenesis [55]. VEGF acts via VEGFR1 and VEGFR2 located in vascular endothelial cells as well as in smooth muscle cells surrounding the vessels.

VEGF family have a prominent role in post- MI angiogenesis VEGF acts through binding to its receptor VEGF receptor 2 (VEGFR2), promoting endothelial cell survival, proliferation, and migration [56] .VEGF is rapidly induced in the ischemic heart in humans not only by hypoxia, but also by mechanical stretch. Another member of the VEGF family of growth factor that preferentially binds to the VEGF Receptor 1 (VEGFR1), Placental Growth Factor (PlGF), also activates angiogenesis in ischemic tissues through two main mechanisms [57].First, PlGF-mediated activation of VEGFR1 leads to transphosphorylation of VEGFR2 and amplification of VEGF-dependent signaling [58] .Second, PlGF promotes the recruitment of proangiogenic VEGFR1 expressing myelomonocytic cells and Sca1 + progenitor cells to ischemic tissues.[59]

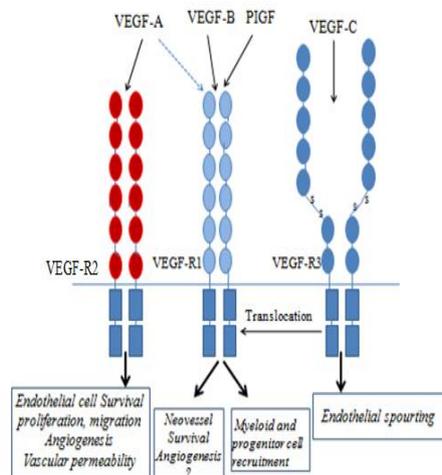


Figure 5: Vascular endothelial growth factor (VEGF) family of growth factors dependent angiogenic pathways.

Ephrin signalling mediated activation of PI3/AKT Pathway as Antiapoptotic & Prosurvival

Apoptosis is programmed cell death which play a fundamental role in development and tissue homeostasis.PI3K/Akt pathway plays a central role in cell cycle progression and apoptosis. The PI3K/Akt signalling pathway regulates cellular metabolism,growth,proliferation, tumour development , metastases and cytoskeletal reorganization. It is part of a complex intracellular cell signalling cascade.PI3K is a plasma membrane-associated protein kinases consisting of three subunits: the regulatory subunits p85 and p55 referred by convention as collectively p85 and a catalytic subunit, p110 [60].Once activated, PI3K catalyzes the phosphorylation of PIP₂ to produce PIP₃. PIP₃ then activates intracellular signalling through its binding to many signalling proteins, including Akt [61].The PI3K/Akt pathway functions downstream of receptor tyrosine kinases (RTKs) as well as independently of RTKs. Non-RTK activation of this pathway may be from other intracellular signalling pathways or from other membrane receptors including G-protein coupled receptors [62]. Its activity is regulated positively by Ras and negatively by Phosphate and tensin homolog (PTEN).

Mammalian target of rapamycin (mTOR) is a major downstream signalling protein involved in protein translation via the eIF4E complex and S6K which is activated by Akt [63]. Cell survival is promoted through antiapoptotic effects, particularly inhibition of the pro-apoptotic Bcl-2 family members BAD and BAX.Further, inhibition of glycogen synthase kinase 3 (GSK-3) increases cellular translation of proteins as does phosphorylation of 4eBP-1.

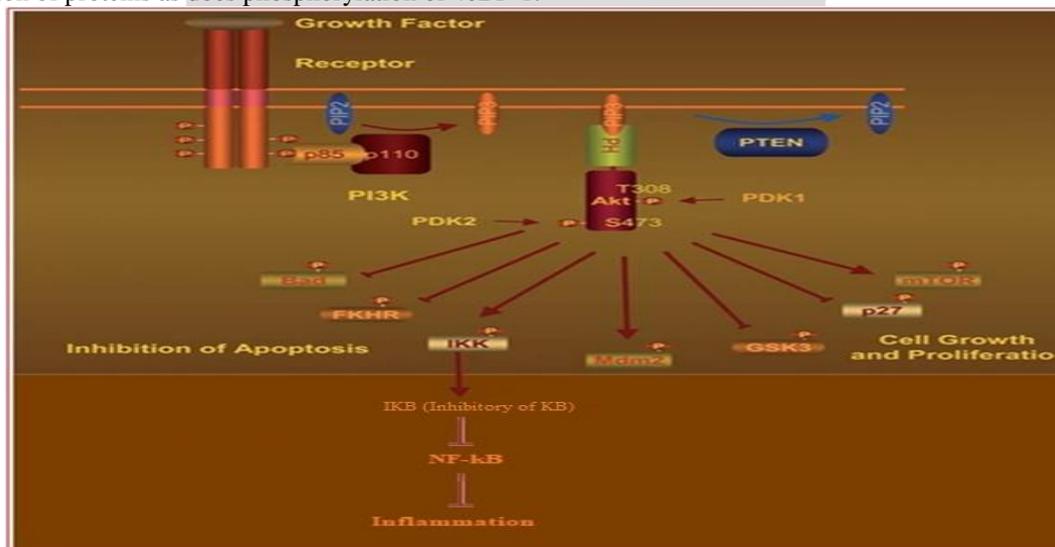


Figure 6: Effects of PI3 / Akt Pathway Activation

Activation of growth factor receptor protein tyrosine kinases results in autophosphorylation on tyrosine residues. PI3K is recruited to the membrane by directly binding to phosphotyrosine consensus residues of growth factor receptors or adaptors through one or both SH2 domains in the adaptor subunit. This leads to allosteric activation of the catalytic subunit. Activation results in production of the second messenger phosphatidylinositol-3,4,5-trisphosphate (PIP3). The lipid product of PI3K, PIP3, recruits a subset of signalling proteins with pleckstrin homology (PH) domains to the membrane, including PDK1 and Akt. PTEN, is a PI-3,4,5-P3 phosphatase, which negatively regulates the PI3K/Akt pathway. Once activated, Akt mediates the activation and inhibition of several targets, resulting in cellular survival, growth and proliferation through various mechanisms.

Regulation of cell growth and survival by Akt also occurs by the NF- κ B pathway via activation of I κ B kinase (IKK). The nuclear factor- κ B (NF- κ B) family of transcription factors control the expression of genes involved in many critical physiological responses such as inflammatory responses, proliferation, differentiation, cell adhesion and apoptosis. NF- κ B signaling pathways can be divided into canonical and non-canonical pathways. In the canonical pathway, I kappa B kinase (IKK) phosphorylates I κ B α (inhibitor of κ B) at two N-terminal serines, triggering its ubiquitination and proteasomal degradation; this leads to the nuclear translocation of NF- κ B. NF- κ B transcription factors have been associated with several aspects of tumorigenesis, including preventing apoptosis, and increasing a tumor's angiogenic and metastatic potential. NF- κ B translocation plays a role in inflammation through induction of

tumorigenesis
metastatic
transcript

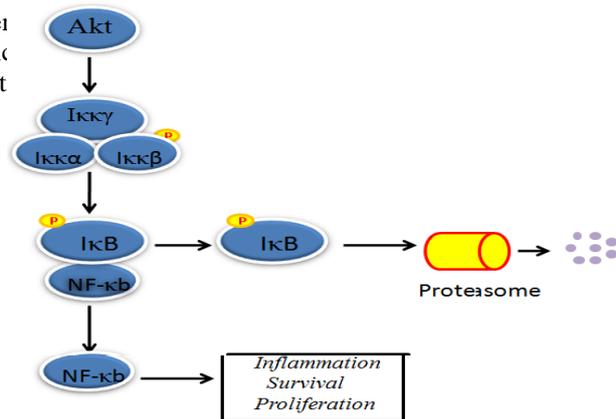


Figure 7: Regulation of NF- κ B by Akt

EphrinA1/EphA receptor expression changes also appear to be involved in regulating pathways involved with apoptosis in endothelial cells [16]. The role for the EphA4 receptor in mediating cell death, and it is reasonable to the activation of the receptor is anti-apoptotic, while inhibition or removal of the receptor is pro-apoptotic [64]. Mice lacking EphA4 have thymic hypoplasia and decreased peripheral T-cells, and this was demonstrated to be due to increased apoptosis and reduced cell cycling [65].

A mice with MI induced by permanent coronary artery occlusion and mRNA levels of the EphB4 receptor and the ephrinB2 ligands in CMs 2 week's post-MI. The ephrinB2 chimeric protein, ephrinB2-Fc, also induced endothelial cell proliferation and sprouting when used *in vitro*. Following MI, mice that were treated with an injection of ephrinB2-Fc had a 28% increase in capillary density in the peri-infarct region [15].

In another study by Dries *et al*, gene Expression of the ephrin A1 protein was seen in cardiomyocytes throughout the myocardium and expression increased post myocardial infarction. Intramyocardial administration of ephrinA1-Fc at the time of ischemia in non-reperfused myocardium results expression of EphA receptors and the ephrinA1 ligands in the healthy murine myocardium and in response to ischemia in nonreperfused myocardium have been reported. In increased EphA1 and EphA2 gene expression and EphA4 protein and increased phosphorylated Akt protein expression, reduced PARP cleavage, indicative of improved cellular survival via reduction in apoptosis was observed. Infarct size was also reduced [11]. Infarct size was reduced by 37% and the volume of the myocardium increased 2-fold with the activation of ephrinA-1.

Ephrin receptor in infarct repair

Several studies implicate the EphA/ephrin-A system in cardioprotection and heart repair by cardiac progenitor cells. Cardiac repair consisted of clusters of closely packed human cardiomyocyte and coronary Vessels. EphA2 is expressed in immature progenitor cells and ephrin-A1 in cardiomyocytes. In rodent models of myocardial infarction, intramyocardial administration of ephrin-A1 Fc reduced heart damage and promoted the migration of progenitor cells to damaged the myocardium. Progenitor cells stimulated with ephrin-A1 Fc before their injection into the injured heart also homed more effectively to damaged regions, and regenerative processes resulting increased myocyte numbers, decreased infarct size and reduced arrhythmic events. On the other hand, EphA2 signaling defects reduced the migratory and regenerative capacity of cardiac progenitor cells, which may impaired the regenerative ability of the aging. Thus, EphA2 function promotes the tissue salvage and improve cardiac repair by transplanted cardiac progenitors for the treatment of myocardial infarction and heart failure.

The role of the ephrin A1-EphA2 axis in myocardial repair was established *in vitro* by exposing the EphA2-positive hCSCs to a human ephrin A1 – Fc chimeric protein which resulted in increased cellular motility. While administration of Ephrin A1 *in vivo* following MI enhanced the migration of hCSC to the damaged myocardium and the initiation of a regenerative response. The ephrin A1 mediated activation of hCSCs, infarct size, the number of newly-formed myocytes, and their volume were compared with the corresponding values in infarcted hearts injected with hCSCs exposed to Fc (human IgG), i.e controls. Following treatment with ephrin A1-hCSCs, cardiac repair resulted in a 37% reduction of infarct size, increase in LV developed pressure (LVDP) and ejection fraction (EF)[13]. These data represent a cardioprotective role for exogenous ephrinA1-Fc administration in

the setting of acute MI and may present a novel therapeutic approach to be used alone or in conjunction with stem/progenitor cells or other biomaterials

Genet *et al.*, 2008 have described a role of ephrinB1 in the murine heart .Although this study did not include an experimental model of MI, their characterization of the expression of ephrinB1 in the murine heart provides further evidence for the role of ephrinB1 in CM stability. The pivotal role for ephrinB1 in cardiac tissue and its capacity to preserve cardiac function during times of stress.

EPHRIN RECEPTORS IN OTHER DISEASES

Nervous system

Eph receptors and ephrins are highly expressed in nervous system, where they regulate the tissue patterning, axon guidance and the formation of synaptic connections[5].Some family members remain substantially expressed in the adult nervous system, where they control the structure and function of synapses and various aspects of neural stem/progenitor cell biology [68,70].The Eph/ephrin system has also been linked to neuropathologies ranging from inhibition of neural repair after traumatic injury and stroke to neurodegenerative diseases and chronic neuropathic pain. EphA4 and EphB2 receptors are highly expressed in several nervous system disorders.

Pain

The EphB/ephrin-B system has been implicated in various types of pain, including chronic neuropathic pain caused by peripheral nerve injury, inflammatory pain and cancer pain [69,73]. The increased activation of postsynaptic EphB receptors (particularly EphB1) in neurons of the spinal cord by well as hyperexcitability of the sensory neurons. EphB signaling potentiates the efficacy of synapses involved in sensing pain and decreases pain threshold levels by enhancing Src-dependent NMDA receptor phosphorylation and calcium currents.

Changes in gene transcription may also play a role in the effects of ephrin-B/EphB signaling on pain. Importantly, reduced EphB1 expression or agents that inhibit EphB-ephrin-B interaction in the spinal cord such as EphB Fc, thermal hyperalgesia, mechanical allodynia and opiate-resistant pain in rodent models. This suggests that antagonists targeting EphB receptors, such as EphB1, A novel class of analgesics for the treatment of difficult-to-control chronic pain. Other studies suggest that the EphA/ephrin-A system may also regulate pain [74].

Atherosclerosis

Ephrin-B2 expressed by inflammatory cells can regulate their interaction with endothelial cells. Therefore, agents blocking EphB/ephrin-B2 could complement current drugs targeting the VEGF system. Moreover, EphrinA1 has *in vivo* angiogenic properties during inflammatory angiogenesis induced by tumour necrosis factor [75]. Additionally, Eph receptors/ephrins expressed in blood vessels and their counterparts in immune cells are involved in inflammatory processes ranging from increased endothelial permeability and inflammatory cell transmigration across the endothelium to atherosclerotic plaque development.

EphrinB2 can activate endothelial cells via the EphA4 receptor leading to increased adhesion of monocytes. The expression of ephrinB2 is increased by pro inflammatory stimuli in human macrophages, which might be an explanation for the increased expression of ephrinB2 in plaque-associated macrophages. The involvement of the ligand ephrinB2 in the process of atherosclerosis was already postulated by other studies. It was shown that endothelial ephrinB2 activates EphB2 receptor on monocytes and induces cytokine expression in monocytes . Analysis of the expression pattern of ephrinB2 in atherosclerotic plaques showed that it is expressed on endothelial cells within the aortic arch, a region known to be predisposed for atherosclerotic lesions [76]. Furthermore, ephrinB2 is up regulated under proatherosclerotic flow conditions and acts as a chemoattractant for leukocyte migration . In vascular biology, Ephs and ephrins classically regulate segregation of endothelial cells into veins (EphB4) and arteries (ephrinB2) during developme [77]. Additionally, both B-class and A-class Ephs and ephrins mediate angiogenesis in the adult. Recent evidence suggests B-class ephrins show altered expression in the atherosclerotic plaque and may mediate monocyte targeting to the plaque .

EphA receptors and ephrinA ligands on the endothelial cell surface function as counter-receptors that stimulate either lymphocyte adhesion or repulsion in a highly cell type specific manner Interestingly, the EphA2 gene resides on a region of chromosome 1 linked to premature myocardial infarction in humans (1p36) and on a region of mouse chromosome 4 linked to enhanced susceptibility to atherosclerosis [78].

Atherosclerotic plaques from humans and hypercholesterolemic mice show robust EphA2 expression in the endothelium and in leukocytes, whereas EphA2 expression is undetectable in non-atherosclerotic vessels. Multiple atherogenic mediators induce endothelial EphA2 expression and enhance EphA2 activation concomitant with expression of its ligand ephrinA1. EphrinA1 co-localizes with EphA2 in the endothelial cell layer of mouse plaques suggesting that EphA2 expressed within the atherosclerotic plaques likely becomes activated.

EphA2 activation with recombinant ephrinA1 stimulates proinflammatory and prothrombotic gene expression as well as enhanced monocyte adhesion, and oxLDL-induced but not cytokine-induced VCAM-1 expression requires EphA2 activation. Taken together, these data suggest that EphA2 activation perpetuates pro inflammatory gene expression and atherosclerosis associated endothelial cell activation[79].

Cancer

Eph receptors and ephrins are expressed in tumors and can affect malignancy through bidirectional signaling pathway [8,81]and have ability to either promote or suppress tumorigenicity. Their expression can increase or decrease during cancer progression due to chromosomal amplification or loss, transcriptional regulation by oncogenic signaling pathways, promoter methylation and microRNAs [8,83].A large amount of studies have revealed a co -relation of ephrin receptors expression with cancer progression

[84]. In particular, EphB2 expression has been observed in both gastric and colon cancers, neuroblastomas, small cell lung carcinoma, and melanoma and in a variety of human cancer cell lines [85]. Although there is a positive correlation between expression of Eph receptor and cancer, it is not clear how the activity of the Eph receptor could contribute to tumor progression. The potential influence of Eph receptors on cell adhesion, motility, guidance, and position has been considered as a mechanism by which they could exacerbate tumorigenesis. Recently, EphB2 and EphB3 were implicated in controlling the segregation and position of cells along the crypt-villus axis in the intestine [7]. This same study identified EphB2 as a target of the wnt signaling pathway, which is hyperactivated by genetic defects associated with the majority of colorectal cancers.

Lung Cancer: EphB3 is also overexpressed in lung cancers, whereas the ephrin-B1 and ephrin-B2 ligands are downregulated [87]. This enhances lung cancer cell migration/invasiveness as well as growth through an EphB3 ligand- and kinase-independent mechanism that remains to be characterized [88]. EphB4 overexpression in lung cancer can also contribute to malignancy. In contrast, the kinase-inactive EphB6 is downregulated in aggressive lung cancers due to promoter hypermethylation and represents a prognostic marker indicating low metastatic potential. EphB6 expression reduced lung cancer cell migration in culture and metastasis in a xenograft model, and these activities were impaired by EphB6 lung cancer mutations [89].

Tumor suppressor, EphA3 expression is also downregulated in a high proportion of lung cancers due to decreased gene copy number [90]. EphA2 and EphA4 are less frequently mutated in lung cancer. High EphA4 expression in patient tumors correlates with improved outcome and EphA4 can inhibit lung cancer cell migration/invasion, suggesting a tumor suppressor role [91].

Brain Cancer: The EphA2 and EphA3 receptors were recently found to promote the self-renewal of glioblastoma stem cells [91]. Moreover, EphA2 serine 897 phosphorylation is elevated in the most aggressive tumors, particularly in the stem cell population, suggesting an important role for EphA2 phosphorylation by AKT in glioblastoma malignancy [29]. Indeed, downregulation of EphA2 or EphA3 expression by RNA interference or administration of high doses of ephrin-A1 Fc drastically reduced glioblastoma xenograft tumorigenicity [94]. Another EphA receptor, EphA4, can promote glioblastoma cell proliferation and migration by potentiating fibroblast growth factor (FGF) receptor oncogenic signaling in an ephrin-dependent manner. Furthermore EphB2, which can be upregulated in glioblastoma as a consequence of decreased microRNA-204 levels, was shown to promote invasiveness while inhibiting proliferation in glioblastoma-derived neurospheres and mouse orthotopic xenografts [93]. Since these effects depend on EphB2 forward signaling, inhibiting EphB2 expression/signaling could help block the infiltration of glioblastoma cells into the brain, but should be accompanied by strategies to inhibit proliferation.

Bladder Cancer: Eph B4/ephrinB2 signaling promotes tumor growth, invasiveness, chemoresistance and tumor angiogenesis in bladder cancer. Eph B4 overexpression is partially regulated by epidermal growth factor receptor (EGFR) signaling and promotes cancer survival through antiapoptosis signaling. In esophageal tumors, the Eph B4 gene appears amplified and contributes to tumor cell survival and migration [96]. Expression of Eph B4 was associated with clinically aggressive disease in gastric and gastroesophageal junction tumors [98].

Colorectal cancer: Eph B4 was shown to not only promote tumor growth, but also tumor-associated angiogenesis [96]. In an Eph B4 knockdown screen of prostate cancer, EPHB4 was shown to regulate integrin b8, a key determinant of prostate cancer invasiveness .

ovarian cancer: Eph B4 expression is associated with poorer survival, and targeting Eph B4 had promising preclinical activity [98]. Overexpression of the Eph B4 receptor and ligand ephrinB2 has also been correlated with poor outcome in several tumors. Targeting Eph B4/ephrinB2 with specific antibodies was found to be effective in animal models of solid tumors [99].

Aging and Skin Diseases

The Eph/ephrin system participates in a number of diseases and aging-associated conditions. These are upregulated in injured tissues, and their activities can hinder certain wound healing processes such as cell growth and movement, but facilitate the inflammatory cell trafficking, angiogenesis and reestablishment of tissue organization [6,101,102]. These are also present in various skin compartments, including the epidermis and hair follicles. EphA2 expressed in keratinocytes controls epidermal differentiation and homeostasis, and its deregulation has been associated with skin diseases such as psoriasis and cancer. Various Eph/ephrin regulate hair growth, with evidence suggesting that loss of ephrin-A3 contributes to androgenic alopecia [102,103]. Thus; the Eph/ephrin systems represent a new target for counteracting effects of aging on hair loss.

Glucose Haemostasis

Role of the EphA/ephrin-A system is the control of glucose homeostasis. EphA forward signaling inhibits insulin secretion in pancreatic β cells when glucose levels are low, while ephrin-A5 reverse signaling promotes insulin secretion in response to elevated glucose [6]. Interestingly, EphA forward signaling induced by ephrin-A5 also plays a complementary role in the glucose-sensing hypothalamic region of the brain, where it promotes the release of hormones that correct hypoglycaemia [104]. Eph receptors and ephrins also regulate bone homeostasis and remodeling. The bone anabolic effects of EphB4 expressed in osteoblasts depend on coordinately promoting osteoblast differentiation and restraining osteoclast precursor differentiation through interactions with ephrin-B2/ephrin-B1 expressed by the two cell types [105]. In contrast, EphA2/ephrin-A2 signaling in osteoblasts/osteoclasts can negatively regulate bone formation.

Thus, Eph/ephrin family members could represent therapeutic targets to treat bone disorders such as arthritis, osteolytic lesions in multiple myeloma, bone-associated metastases and osteoporosis.

Doxazosin as Ephrin A Receptors Agonist

During identification of small molecules agonist of EphA receptor molecule modelling EpA-2 ligand binding domain revealed that binding pocket of EphA2 can incorporate up to 4 amino acids, suggesting that it could accommodate small molecules with a molecular weight (MW) of about 500 Dalton [106]. The size falls in the range of common drugs [107]. Many compounds were screened for their ability to induce EphA2 activation in MDAMB-231 breast cancer cells. MDA-MB-231 cells express endogenous EphA2 as well as other Eph receptors; Doxazosin was identified as a novel agonist of EphA2 receptors.

Doxazosin, a piperazinyl quinazoline compound, is a long lasting selective inhibitor of α_1 -adrenergic receptors. Doxazosin is an FDA-approved drug (Cardura H) commonly prescribed treatment for patients with hypertension [17-18] and enlarged prostate. Doxazosin effectively lowers blood pressure by blocking nor epinephrine binding to α_1 -adrenergic receptors, relaxing smooth muscle cells, subsequently decreasing vascular tone and reducing peripheral vascular resistance. Doxazosin is also used to treat urinary retention problems in patients with benign prostatic hyperplasia blocking the action of α_1 -adrenergic receptors, doxazosin treatment leads to relaxation of the smooth muscles surrounding the prostate, which ease urine flow and decrease bladder outlet obstruction. In addition, doxazosin has shown a beneficial effect on lipids by modestly lowering both total cholesterol and triglycerides. Doxazosin (maximum total daily dose 0.5 mg to 16 mg) in 271 completed clinical studies for hypertension or benign prostatic hyperplasia (BPH). Doxazosin activated EphA2 receptor in a dose-dependent manner at 25 μ M and became stronger at 50 μ M or higher [109].

Doxazosin induces catalytic activation of EphA2 independent of α_1 -adrenoreceptor antagonism in MDA-231-A2 cells. As pretreatment of MDA-231-A2 cells with the well-characterized irreversible α_1 -adrenoreceptor inhibitor, phenoxybenzamine results in induction of EphA2 phosphorylation by doxazosin. MDA-231-A2 cells were chosen because they have previously been shown to express both α_1a and α_1b -adrenoreceptors [110].

Doxazosin activates EphA2 receptor in different cell types. In Human embryonic kidney 293 cells over expressing EphA2 (HEK 293-A2) significant activation of EphA2 was seen upon treatment with doxazosin starting at 25 μ M. Further, in PC-3 cells that express high levels of endogenous EphA2 receptor 111,112. Doxazosin also activated endogenous EphA2 in PC-3 cells, although it was not evident until 50 mM. In addition, EphA2 activation in PC-3 cells further supports the α_1 -adrenoreceptor-independent mechanism, as these cells lack detectable 1α -adrenoreceptor expression [113].

In Human embryonic kidney 293 cell lines over expressing EphA1, EphA2, EphA3, EphA4, and EphB3 kinases doxazosin activated both EphA2 and EphA4 kinases following a similar dose-response relationship. However, no activation of EphA1 was seen at the same concentrations, and activation of EphA3 was weak compared to that of EphA2 and EphA4.

CONCLUSION

Eph receptors and their *ligands Eph* are largest family of receptor tyrosine kinases Eph-A receptors and ephrin-A1 ligand are expressed in healthy myocardium and expression increased post myocardial infarction. Intramyocardial administration of ephrinA1-Fc at the time of ischemia in non-reperfused myocardium results in increase in expression of Eph-A1 and Eph-A2 receptors, EphA4 protein and increased phosphorylated Akt protein expression. Activation of PI3K/Akt-dependent signaling has been shown to prevent cardiac myocyte apoptosis and protect the myocardium from I/R injury. Akt promotes survival of cardiomyocytes in vitro and protects against ischemia-reperfusion injury in mouse heart.

Doxazosin has been reported to activate EphA2 receptors in HEK and PC-3 cells respectively. In present study we employed doxazosin to explore the role of ephrin A2 receptors in cardioprotective effect against myocardial ischemia reperfusion injury. Doxazosin at different doses significantly attenuated ischemia and reperfusion induced increase in myocardial infarct size, release of LDH and CK. Oxidative stress generated within first few minutes of myocardial reperfusion has been reported in myocardial injury and cardiomyocyte death through a number of different mechanisms. Doxazosin at 25 μ M and 50 μ M doses significantly attenuated ischemia and reperfusion induced increase in TBARS as well as up-regulate ROS-detoxifying enzymes Glutathione and Catalase. In recent studies, it has been reported that PI3/Akt pathway has been implicated in protection against oxidative stress by regulating activity of Nrf2 (Nuclear factor erythroid 2-related factor (Nrf2), a master regulator of antioxidant defense gene and a master transcription factor released during oxidative stress. Thus, our investigation indicates that PI3/Akt pathway may be involved in cardioprotective effect of Doxazosin downstream Ephrin A2 receptor activation.

Reference

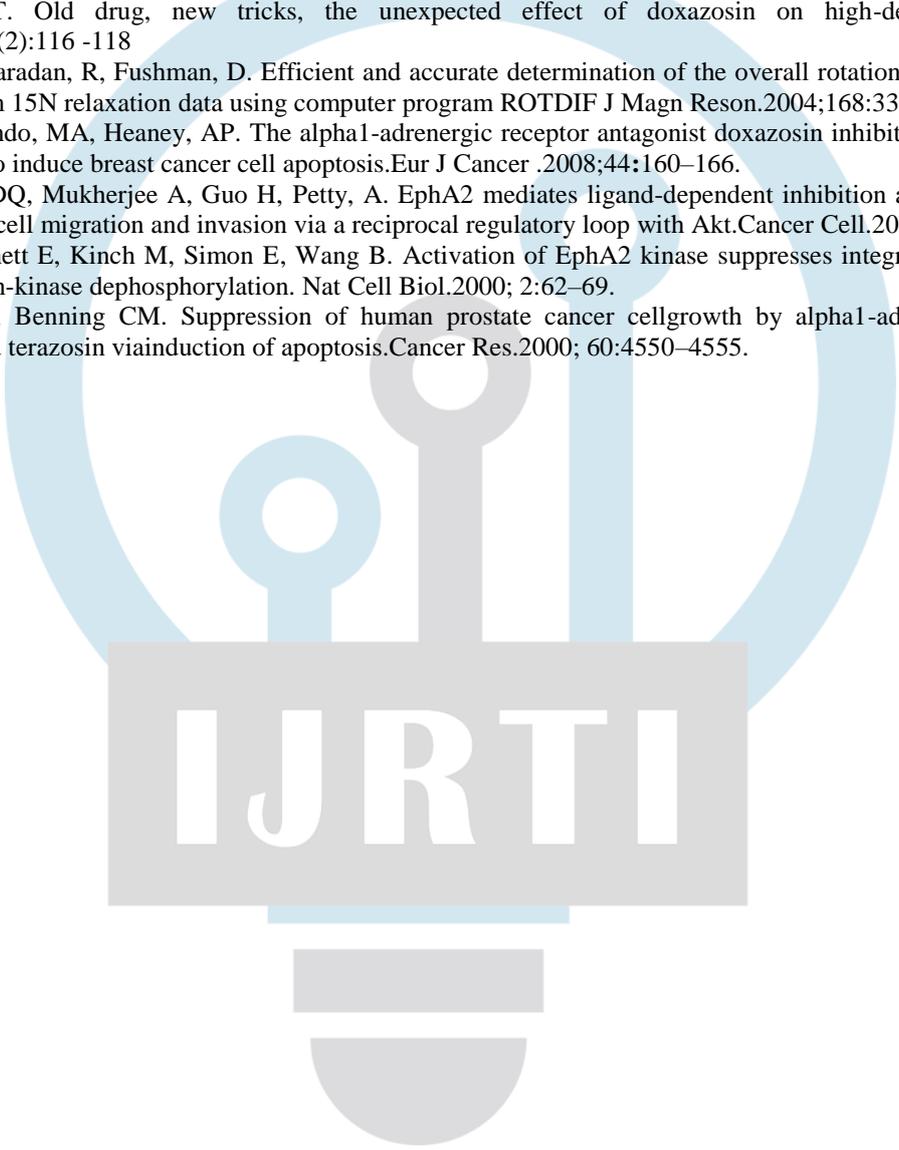
1. Eriksson M, Asplund, K, and Svedlund, M. Thoughts about and Expectation of Their Future Life after the Patient's Hospital discharge Following Acute myocardial infarction. 2010; 3485-3493.
2. Stricker, BH, Stijnen, T. Analysis of individual drug use as a time-varying determinant of exposure in prospective population-based cohort studies. Eur J Epidemiol. 2010; 25(4): 245-251.
3. Braunwald, E. Myocardial reperfusion - a double-edged sword. J Clin Invest. 1985; 76:1713-1719.
4. Piper, HM, Abdallah, Y, Schafer, C. The first minutes of reperfusion: a window of opportunity for cardioprotection. Cardiovasc Res. 2007; 61: 365-71.
5. Pasquale, E.B. Eph receptor signalling casts a wide net on cell behaviour. Nat. Rev. Mol. Cell Biol. 2005; 6: 462-475.
6. Pasquale, E.B. Eph-ephrin bidirectional signaling in physiology and disease. Cell. 2008; 133:38-52.
7. Battle, E, Wilkinson, DG. Molecular mechanisms of cell segregation and boundary formation in development and tumorigenesis", Cold Spring Harbor Perspect Biol. 2002; 4.a008227.
8. Pasquale, EB. Eph receptors and ephrins in cancer Bidirectional signalling and beyond. Nat Rev Cancer. 2010; 10:165-180.

9. Goldsmith, A.D, Sarin, S, Lockery, S, Hobert, O. Developmental control of lateralized neuron size in the nematode *Caenorhabditis elegans*. *Neural Dev.*2010;5:33.
10. Arvanitis, DN, Davy, A. "Regulation and misregulation of Eph/ephrin expression. *Cell Adh Migr.*2012;6:131–7.
11. Dries, J.L, Kent, S.D, and Virag, J.A. Intramyocardial administration of chimeric ephrinA1-Fc promotes tissue salvage following myocardial infarction in mice. *J. Physiol.*2012.589 (7):1725–1740.
12. Virag, JA, Lust, RM, Coronary artery ligation and intramyocardial injection in a murine model of infarction. *J Vis Exp.*2011;52: 2581.
13. Goichberg, P, Bai, Y. The ephrinA1–EphA2 system promotes cardiac stem cell migration after infarction. *Circ Res*2011;108: 1071–1083.
14. Munoz, JJ, Alfaro, D, Garcia-Ceca, J, Alonso, CL, Jimenez, E, Zapata, A. Thymic alterations in EphA4-deficient mice. *J Immunol.*2006;177 :804–813.
15. Mansson-Broberg, A, Siddiqui, AJ, Genander, M. (2008). Modulation of ephrinB2 leads to increased angiogenesis in ischemic myocardium and endothelial cell proliferation. *Biochem Biophys Res Commun.*2008;373:355–359.
16. Wesley, T, O'Neal, et al. Ephrin–Eph signaling as a potential therapeutic target for the treatment of myocardial infarction. *Am J Pathol.*2013;15: 433–440.
17. Black, H. R. Doxazosin as combination therapy for patients with stage 1 and stage 2 hypertension. *J. Cardiovasc. Pharmacol.*2003;866–869
18. Ceral, J, Solar, M. Doxazosin: safety and efficacy in the treatment of resistant arterial hypertension. *Blood Press.*2009;18:74–77.
19. Clarke, D. E. Alpha adrenoceptor blockade in the treatment of benign prostatic hyperplasia: past, present and future *Br. J. Urol* 1998; 82:167.
20. Tuzi NL, Gullick WJ. Eph, the largest known family of putative growth factor receptors. *Br J Cancer.*1994;69:417–21.
21. Pasquale EB. Eph-ephrin bidirectional signaling in physiology and disease. *Cell.* 2008;133:38–52.
22. Battle E, Wilkinson DG. Molecular mechanisms of cell segregation and boundary formation in development and tumorigenesis. *Cold Spring Harb Perspect Biol.*2012;4:a008227.
23. Lisabeth EM, Falivelli G, Pasquale EB. Eph receptor signaling and ephrins. *Cold Spring Harb Perspect Biol.*2013;5:a009159.
24. Hirai H, Maru Y, Hagiwara K, Nishida J, Takaku F. A novel putative tyrosine kinase receptor encoded by the eph gene. *Science.* 1987;238:1717–20.
25. Pasquale EB. Eph-ephrin promiscuity is now crystal clear. *Nat Neurosci.* 2004;7: 417–418.
26. Pasquale EB. Eph receptor signalling casts a wide net on cell behaviour. *Nat Rev Mol Cell Biol.* 2005;6: 462–475
27. Pasquale EB. Eph receptors and ephrins in cancer: Bidirectional signalling and beyond. *Nat Rev Cancer.*2010; 10: 165–180.
28. Miro-Casas, E, Ruiz-Meana, M, Agullo, E, Stahlhofen, S, Heusch, G, Garcia-Dorado, D. Connexin43 in cardiomyocyte mitochondria contributes to mitochondrial potassium uptake. *Cardiovasc Res.*2009;83:747–756.
29. Hori, M, Nishida, K. Oxidative stress and left ventricular remodelling after myocardial infarction. *Cardiovascular research.*2009;81:457–64.
30. Walker, O, Varadan, R, Fushman, D. Efficient and accurate determination of the overall rotational diffusion tensor of a molecule from ¹⁵N relaxation data using computer program ROTDIF. *J Magn Reson.*2009;168: 336–345.
31. Yellon, DM, Hausenloy, DJ. Myocardial reperfusion injury. *N Engl J Med.*2007;357(11): 1121–1135
32. Hendrikx, M, Mubagwa, K, Verdonck, F, Overloop, K, Van Hecke, P, Vanstapel, F. New Na(+)-H + exchange inhibitor HOE 694 improves postischemic function and high-energy phosphate resynthesis and reduces Ca²⁺ + overload in isolated perfused rabbit heart. *Circulation.*1994;89:2787–98. .
33. Inserte, J, Barba, I, Hernando, V, Garcia-Dorado, D. Delayed recovery of intracellular acidosis during reperfusion prevents calpain activation and determines protection in postconditioned myocardium. *Cardiovasc Res.*2009;81:116–22.
34. Bowers, KC, Allshire, AP, and Cobbold, PH. Bioluminescent measurement in single cardiomyocytes of sudden cytosolic ATP depletion coincident with rigor. *J Mol Cell Cardiol.*1992;24:213–8
35. Piper, HM, Garcia-Dorado, D, Ovize, M. A fresh look at reperfusion injury. *Cardiovasc Res.*1998;38(2):291–300.
36. Halestrap, AP, Clarke, SJ, Javadov, SA. Mitochondrial permeability transition pore opening during myocardial reperfusion—a target for cardioprotection. *Cardiovasc Res.*2004;61:372–385.
37. Skyschally, A, Van Caster, P, Boengler, K, Gres, P, Musiolik, J, Schilawa, D. Ischemic postconditioning in pigs: no causal role for RISK activation. *Circ Res.*2009;104:15–18
38. , William, F, Griffin. Ephrin–Eph signaling as a potential therapeutic target for the treatment of myocardial infarction. *Am J Pathol.*2013;15:433–440.
39. Chrencik, JE, Brooun, A, Recht, MI, Kraus, ML, Koolpe, M, Kolatkar, AR, Bruce, RH, Martiny-Baron, G, Widmer, H, Pasquale, EB, and Kuhn, P. Structure thermodynamic characterization of the EphB4/Ephrin-B2 antagonist peptide complex reveals the determinants for receptor specificity. *Structure.*2006;14:321–330.
40. Pasquale, EB. Eph receptors and ephrins in cancer Bidirectional signalling and beyond. *Nat Rev Cancer.*2010; 10:165–180.

41. Murai, KK, Pasquale, EB. Eph'ectivesignaling: forward, reverse crosstal J. Cell Sci.2003; 116: 2823–2832
42. Egea, J, and Klein, R. Bidirectional Eph-ephrinsignaling during axon guidance. Trends Cell Biol.2007; 17:230–238.
43. Freywald, A0, Sharfe, N, Miller, CD, Eph A receptors inhibit anti-CD3-induced apoptosis in thymocytes. J. Immunol.2006;176 :4066-4074.
44. Sancho, E, Battle, E, Clever, H, Live and let die in the intestinal epithelium. Curr Opin Cell Biol.2003;15:763–70.
45. Wang, SD, Rath, P, Lal, B, Richard, JP, Li, Y. EphB2 receptor controls proliferation/migration dichotomy of glioblastoma by interacting with focal adhesion kinase. Oncogene.2012;31:5132–43
46. Battegay, E.J, Angiogenesis: mechanistic insights, neovascular diseases, and therapeutic prospects. J Mol. Med.1995; 73:333–346
47. Khurana, R, Martin, J, Zachary, I. Role of angiogenesis in cardiovascular disease. A critical appraisal. Circulation.2005;112 :1813-1824.
48. Shiojima, I, Sato, K, Izumiya, Y, Schiekofer, S, Ito, M, Liao, R, Colucci, WS, and Walsh, K. Disruption of coordinated cardiachypertrophy and angiogenesis contributes to the transition to heart failure. J Clin Invest.2005;115: 2108–2118.
49. Pugh, CW, Ratcliffe, PJ. Regulation of angiogenesis by hypoxia: role of the HIF system. Nat Med.2003;(9):677–684.
50. Jurgensen, JS, Rosenberger, C, Wiesener, MS, Warnecke, C, Horstrup, JH, Grafe, M, Philipp, S, Griethe, W, Maxwell, PH, Frei, U, Bachmann, S, Willenbrock, R, Eckardt, KU. Persistent induction of HIF-1 α and -2 α in cardiomyocytes and stromal cells of ischemic myocardium. FASEB J.2004;18:1415–1417.
51. Kido, M, Du, L, Sullivan, CC, Li, X, Deutsch, R, Jamieson, SW, and Thistlethwaite, PA. Hypoxia-inducible factor 1- α reduces infarction and attenuates progression of cardiac dysfunction after myocardial infarction in the mouse. J Am Coll Cardio.2005; 146:2116–2124
52. Skuli, N, Majmundar, AJ, Krock, BL, Mesquita, RC, Mathew, LK, Quinn, ZL, Runge, A, Liu, L, Kim, MN, Liang, J, Schenkel, S, Yodh, AG, Keith, B, and Simon, MC. Endothelial HIF-2 α regulates murine pathological angiogenesis and revascularization processes. J Clin Invest.2012; 12: 1427–1443.
53. Stein, E, Lane, AA, Cerretti, DP, Schoecklmann, HO, Schroff, AD, Van, Etten, RL, Daniel, TO. Eph receptors discriminate specific ligand oligomers to determine alternative signaling complexes, attachment and assembly responses. Genes Dev.1998;12:667–678.
54. Cheng, N, Brantley, D. M, and Chen, J. The ephrins and Eph receptors in angiogenesis", Cytokine Growth Factor Rev.2002;13:75–85.
55. Ferrara, N, Gerber, HP, and LeCouter, J. The biology of VEGF and its receptors. Nat Med.2003; 9:669–676
56. Li, J, Hampton, T, Morgan, JP, and Simons, M. Stretch-induced VEGF expression in the heart. J Clin Invest .1997;100:18–24
57. Autiero, M, Waltenberger, J, Communi, D, Kranz, A, Moons, L, Lambrechts, et al. Role of PlGF in the intra- and intermolecular cross talk between the VEGF receptors Flt1 and Flk1. Nat Med.2003;9:936–943.
58. Lyngbaek, S, Schneider, M, Hansen, JL, and Sheikh, SP. Cardiac regeneration by resident stem and progenitor cells in the adult heart. Basic Res Cardiol.2007;102:101–114.
59. Martini, M, Ciraolo, E, Gulluni, F, and Hirsch, E. Targeting PI3K in cancer: any good news. Front Oncol.2013;3:8.
60. Jiang, X, Chen, S, Asara, JM, and Balk, SP. Phosphoinositide 3-kinase pathway activation in phosphate and tensin homolog (PTEN)-deficient prostate cancer cells is independent of receptor tyrosine kinases and mediated by the p110 β and p110 δ catalytic subunits. J Biol Chem.2010;28:14980-14989.
61. Drake, JM, Graham, NA, Lee, JK. Metastatic castration resistant prostate cancer reveals intrapatient similarity and interpatient heterogeneity of therapeutic kinase targets. Proc Natl Acad Sci USA.2013;110:4762-4769.
62. Kremer, CL, Klein, R.R, Mendelson, J. Expression of motor signaling pathway markers in prostate cancer progression. Prostate.2006;66:1203-1212.
63. Hubbard, SR. Uptake of membrane autophagy in receptor tyrosine kinases. Nat Rev Mol Cell Biol.2004;5:464–71.
64. Munoz, JJ, Alfaro, D, Garcia-Ceca, J, Alonso, CL, Jimenez, E, Zapata, A. Thymic alterations in EphA4-deficient mice. J Immunol.2006;177 :804–813.
65. Dries, J.L, Kent, S.D, and Virag, J.A. Intramyocardial administration of chimeric ephrinA1-Fc promotes tissue salvage following myocardial infarction in mice. J. Physiol.2011;589 (7):1725–1740.
66. Goichberg, P., Bai, Y. The ephrinA1–EphA2 system promotes cardiac stem cell migration after infarction. Circ Res.2011;108:1071–1083.
67. Genet, G, Guilbeau-Frugier, C, Honton, B. Ephrin-B1 is a novel specific component of the lateral membrane of the cardiomyocyte and is essential for the stability of cardiac tissue architecture cohesion. Circ Res.2012;110:688–700.
68. Sheffler-Collins, SI, Dalva, MB. EphBs: an integral link between synaptic function and synaptopathies. Trends Neurosci.2012;(35):293–304.
69. Chen, Y, Fu, AK, and Ip, NY. Eph receptors at synapses: implications in neurodegenerative diseases. Cell Signal .2012 ;24:606–11.
70. Conway, A, Vazin, T, Spelke, DP, Rode, NA, Healy, KE. Multivalent ligands control stem cell behaviour in vitro and in vivo. Nat Nanotechnol.2013;8:831–8.
71. Cibert-Goton, V, Yuan, G, Battaglia, A, Fredriksson, S, Henkemeyer, M. Involvement of EphB1 receptors signalling in models of inflammatory and neuropathic pain. PLoS ONE.2013;8:e53673.

72. Leandro-Garcia, LJ, Inglada-Perez, L, Pita, G, Hjerpe, E, Leskela. Genome-wide association study identifies ephrin type A receptors implicated in paclitaxel induced peripheral sensory neuropathy. *J Med Genet.* 2013; 50:599–605.
73. Pandey, A, Shao, H, Marks, RM, Polverini, PJ, Dixit, VM. Role of B61, the ligand for the Eckerceptor tyrosine kinase in TNF-alpha-induced angiogenesis. *Science.* 1995; 268:567–569.
74. Braun, J, Hoffmann, SC, Feldner, A, Ludwig, T, Henning, R, Hecker, M, Korff, T. Endothelial ephrin B2-dependent activation of monocytes in arteriosclerosis. *Arteriosclerosis, thrombosis, and vascular biology.* 2011;31:297–305.
75. Wang, HU, Chen, ZF, Anderson, DJ. Molecular distinction and angiogenic interaction between embryonic arteries and veins revealed by ephrin-B2 and its receptor Eph-B4. *Cell.* 1998;93:741–753.
76. Braun, J, Hoffmann, SC, Feldner, A, Ludwig, T, Henning, R, Hecker, M, Korff, T. Endothelial ephrin B2-dependent activation of monocytes in arteriosclerosis. *Arteriosclerosis, thrombosis, and vascular biology.* 2011;31:297–305.
77. Sakamoto, A, Ishibashi-Ueda, H, Sugamoto, Y, Higashikata, T, Miyamoto, S, Kawashiri, MA, Yagi, K, Konno, T, Hayashi, K, Fujino, N, Ino, H, Takeda, Y, Yamagishi, M. Expression and function of ephrin-B1 and its cognate receptor EphB2 in human atherosclerosis: from an aspect of chemotaxis. *Clin Sci (Lond).* 2008;114: 643–650.
78. Welch, CL, Bretschger, S, Latib, N, Bezouevski, M, Guo, Y, Pleskac, N, Liang, CP, Barlow, C, Dansky, H, Breslow, JL, Tall, AR. Localization of atherosclerosis susceptibility loci to chromosomes 4 and using the Ldlr knockout mouse model. *Proc Natl Acad Sci U S A.* 2001;98:7946–7951.
79. Steven, Daniel, Funk, B.S.b, Arif, Yurdagul Jr B.S.a, Patrick Alberta, James, G, Traylor, J. EphA2 Activation Promotes the Endothelial Cell Inflammatory Response: a potential role in atherosclerosis. *Arterioscler Thromb Vasc Biol.* March. 2012;32(3):686–695.
80. Arvanitis, DN, Davy, A. Regulation and misregulation of Eph/ephrin expression. *Cell Adh Migr.* 2012;6:131–7..
81. Nishimura, M, Jung, EJ, Shah, MY, Lu, C, Spizzo, R. Therapeutic synergy between microRNA and siRNA in ovarian cancer treatment. *Cancer Discov.* 2013;3:1302–15.
82. Dodelet, V. C, and Pasquale, E. B. Eph receptors and ephrin ligands: embryogenesis to tumorigenesis. *Oncogene.* 2002;19:5614–5619.
83. Vogt, T, Stolz, W, Welsh, J, Jung, B, Kerbel, R. S. Kobayashi, H, Landthaler, M, and McClelland, M. Overexpression of Lerk-5/Eplg5 messenger RNA: a novel marker for increased tumorigenicity and metastatic potential in human malignant melanomas. *Clin. Cancer Res.* 1998;4:791–797.
84. Batlle, E, Henderson, J. T, Beghtel, H, van den Born, M. M, Sancho, E, Huls, G, Meeldijk, J, Robertson, J, van de Wetering, M, Pawson, T. Catenin and TCF mediate cell positioning in the intestinal epithelium by controlling the expression of EphB/ephrinB. *Cell.* 2020; 111:251–263.
85. Ji XD, Li G, Feng YX, Zhao JS, Li JJ. EphB3 is overexpressed in non-small-cell lung cancer and promotes tumor metastasis by enhancing cell survival and migration. *Cancer Res.* 2012;71:1156–66
86. Li, G, Ji, XD, Gao, H, Zhao, JS, Xu, J. EphB3 suppresses non-small-cell lung cancer metastasis via a PP2A/RACK1/Akt signalling complex. *Nat Commun.* 2012;3:667.
87. Bulk, E, Yu, J, Hascher, A., Koschmieder, S, Wiewrodt, R. Mutations of the EPHB6 receptor tyrosine kinase induce a pro-metastatic phenotype in non-small cell lung cancer. *PLoS ONE.* 2012;7:e44591.
88. Zhuang, G, Song, W, Amato, K, Hwang, Y, Lee, K. Effects of cancer-associated EPHA3 mutations on lung cancer. *J Natl Cancer Inst.* 2012;104:1182–97.
89. Saintigny, P, Peng, S, Zhang, L, Sen, B., Wistuba, II. Global Evaluation of Eph receptors and ephrins in lung adenocarcinomas identifies EphA4 as an inhibitor of cell migration and invasion. *Mol Cancer Ther.* 2012;11:2021–32.
90. Miao, H, Gale, NW, Guo, H, Qian, J, Petty, A. EphA2 promotes infiltrative invasion of glioma stem cells in vivo through cross-talk with Akt and regulates stem cell properties” *Oncogene.* 2014
91. Miao, H, Li, DQ, Mukherjee, A, Guo, H, Petty, A. EphA2 mediates ligand-dependent inhibition and ligand-independent promotion of cell migration and invasion via a reciprocal regulatory loop with Akt. *Cancer Cell.* 2014;16:9–20.
92. Day BW, Stringer BW, Al-Ejeh F, Ting MJ, Wilson J. EphA3 Maintains Tumorigenicity and Is a Therapeutic Target in Glioblastoma Multiforme. *Cancer Cell.* 2013;23:238–48.
93. Wang SD, Rath P, Lal B, Richard JP, Li Y. EphB2 receptor controls proliferation/migration dichotomy of glioblastoma by interacting with focal adhesion kinase. *Oncogene.* 2012;31:5132–43
94. Knoll B, and Drescher, U. Ephrin-As as receptors in topographic projections. *Trends Neurosci.* 2002;25:45–149.
95. Frisen J, Holmberg J, and Barbacid M. Ephrins and their Eph receptors: multitasking directors of embryonic development. *EMBO J.* 1999;18 :5159–5165.
96. Coulthard M. G, Duffy S, Down M, Evans B, Power M, Smith F, Stylianou C, Kleikamp S, Oates A, Lackmann M, Burns G. F. The role of the Eph- *Biol.* 2002;46:375–384.
97. Frisen J, Holmberg J, and Barbacid, M. Ephrins and their Eph receptors: ephrin signalling system in the regulation of developmental patterning. *Int. J. Dev. Biol.* multitasking directors of embryonic development. *EMBO J.* 1999;18:5159–5165.
98. Cowan and Henkemeyer. M. Ephrins in reverse, park and drive. *Trends Cell Biol.* 2002;12:339–346.
99. Wilkinson, D. G. Eph receptors and ephrins: regulators of guidance and assembly”, *Int. Rev. Cytol.* 2000;196:177–244.
100. Batlle E, Wilkinson DG. Molecular mechanisms of cell segregation and boundary formation in development and tumorigenesis. *Cold Spring Harbor Perspect Biol.* 2012;4:a008227.
101. Funk SD, Orr AW. Ephs and ephrins resurface in inflammation, immunity, and atherosclerosis. *Pharmacol Res.* 2013;67:42–52.

- 102.Li, G, Ji, XD, Gao H, Zhao JS, Xu JF. EphB3 suppresses non-small-cell lung cancer metastasis via a PP2A/RACK1/Akt signalling complex. *Nat Commun.*2013;3:667.
- 103.Gordon K, Kochkodan JJ, Blatt H, Lin SY Kaplan N. Alteration of the EphA2/Ephrin-A signaling axis in psoriatic epidermis. *J Invest Dermatol.*2013;133:712.
- 104.Szepietowska, B, Zhu W, Czyzyk J, Eid T, Sherwin RS. EphA5-EphrinA5 interactions within the ventromedial hypothalamus influence counterregulatory hormone release and local glutamine/glutamate balance during hypoglycaemia. *Diabetes.*2013;62: 1282–8.
- 105.Matsuo K, Otaki N. Bone cell interactions through Eph/ephrin: bone modeling, remodeling and associated diseases. *Cell AdhMigr.*2012;6:148–56.
- 106.Myshkin E, Wang B. Chemometrical classification of Ephrin ligands and Eph kinases using GRID/CPCA approach. *J Chem Inf Comput Sci.*2003;43:1004– 1010.
- 107.Lipinski, CA. Drug-like properties and the causes of poor solubility and poor permeability”, *J Pharmacol Toxicol Methods* .2007; 44:235–249.
- 108.Remaley, AT. Old drug, new tricks, the unexpected effect of doxazosin on high-density lipoprotein. *Circ Res.*2007;101(2):116 -118
- 109.Walker, O, Varadan, R, Fushman, D. Efficient and accurate determination of the overall rotational diffusion tensor of a molecule from 15N relaxation data using computer program ROTDIF *J Magn Reson.*2004;168:336–345.
- 110.Hui H, Fernando, MA, Heaney, AP. The alpha1-adrenergic receptor antagonist doxazosin inhibits EGFR and NF-kappa B signalling to induce breast cancer cell apoptosis. *Eur J Cancer* .2008;44:160–166.
- 111.Miao H, Li, DQ, Mukherjee A, Guo H, Petty, A. EphA2 mediates ligand-dependent inhibition and ligand-independent promotion of cell migration and invasion via a reciprocal regulatory loop with Akt. *Cancer Cell.*2009;16:9–20.
- 112.Miao H, Burnett E, Kinch M, Simon E, Wang B. Activation of EphA2 kinase suppresses integrin function and causes focal-adhesion-kinase dephosphorylation. *Nat Cell Biol.*2000; 2:62–69.
- 113.Kyprianou N, Benning CM. Suppression of human prostate cancer cell growth by alpha1-adrenoceptor antagonists doxazosin and terazosin via induction of apoptosis. *Cancer Res.*2000; 60:4550–4555.

A large, light blue watermark logo is centered on the page. It features a stylized lightbulb shape with a circular top and a semi-circular base. Inside the circle, there are three vertical lines of varying heights, each ending in a small circle. Below the circle is a grey rectangular box containing the text 'IJRTI' in white, bold, sans-serif capital letters. Below the box is a grey semi-circular shape, and at the very bottom is a grey semi-circular shape pointing downwards.

IJRTI