Regulation of Neovascularization by *S*-glutathionylation via Wnt5a-sFlt-1 pathway

Colin E Murdoch[1*] Markus M. Bachschmid[2], and Reiko Matsui[2]

[1] Vascular Medicine Section, Aston Medical School, Aston University
Aston Triangle, Birmingham, UK. B47 ET
Vascular Biology Section, Boston University Medical School, Boston, MA, USA 02118.
* Corresponding Author email. c.murdoch@aston.ac.uk

Abstract

S-glutathionylation occurs when reactive oxygen or nitrogen species react with protein cysteine thiols. Glutaredoxin-1 (Glrx) is a cytosolic enzyme which enzymatically catalyzes the reduction of *S*-glutathionylation, conferring reversible signaling function to proteins with redox-sensitive thiols. Glrx can regulate vascular hypertrophy and inflammation by regulating activity of NF-κB and actin polymerization. VEGF-induced endothelial cell (EC) migration is inhibited by Glrx overexpression. In mice overexpressing Glrx, blood flow recovery, exercise function and capillary density were significantly attenuated after hind limb ischemia (HLI). Wnt5a and sFlt-1 were enhanced in the ischemic limb muscle and plasma respectively from Glrx TG mice. A Wnt5a-sFlt-1 pathway had been described in myeloid cells controlling retinal blood vessel development. Interestingly, a Wnt5a-sFlt-1 pathway was found also to play a role in EC to inhibit network formation. *S*-glutathionylation of NF-κB components inhibits its activation. Up-regulated Glrx stimulated Wnt5a-sFlt-1 pathway through enhancing NF-κB signaling. These studies show a novel role for Glrx in post-ischemic neovascularization, which could define a potential target for therapy of impaired angiogenesis in the pathological conditions including diabetes.

Introduction

Imbalance in redox homeostasis is a hallmark of cardiovascular disease, particularly associated with diabetes. Enhanced levels of reactive oxygen and nitrogen species (RONS) act as deteriorating factors under pathophysiological settings [1–3], but are now also recognized as important redox signaling molecules[4,5]. The central way that RONS regulate intracellular signaling is by modulating redox-sensitive cysteine residues. The unique property of the cysteine thiol allows diverse reversible and irreversible oxidative post-translational modifications (Ox-PTM) (Figure 1), resulting in regulatory mechanisms of cellular signaling. With the greater appreciation of redox signaling occurring during physiological and pathological conditions it is becoming apparent that we need to further understand Ox-PTMs [6]. Cardiovascular research has been leading the way to understand the intricate control of redox signaling [7,8].

Cysteine - the redox switch

A pivotal site for Ox-PTMs resides at a terminal thiol (-SH) on the side chain of cysteine residues. The thiol gives rise to the thiolate anion ($R\text{-}S^-$) to generate different oxidative states in reaction with RONS. Some of the modified states such as *S*-nitrosylation and sulfenylation are generally unstable and can be further modified to other Ox-PTMs including *S*-glutathionylation [9]. Some modifications are reversible; *S*-glutathionylation is reduced by Glrx, and protein disulfide is usually reversed by thioredoxin (Trx). On the other hand, higher level of oxidants can modify further the intermediates to sulfinic and sulfonic acid which are irreversible and detrimental to protein function, thus under these settings high oxidative conditions may accelerate disease progression [10]. Taken together the redox sensitive cysteines provide the intracellular mechanistic switch to sense fluctuations of redox levels controlling protein activity [11]. Moreover, this provides the intriguing potential that Ox-PTMs such as *S*-glutathionylation may actually protect proteins from irreversible oxidation [9,12].

Predicting which cysteine residues are redox sensitive is complex [13], the reactivity of the cysteine depends on the redox-milieu local to the cysteine, the reactivity of individual cysteine itself and the neighboring amino acid residues [14]. Databases have been compiled in different cardiovascular tissues to identify which cysteine residues act as a redox switch [10]. Additional studies along similar lines will be required to fully map and appreciate the redox potential of cysteines.

Assessment of *S*-glutathionylation

The “biotin switch assay” described previously provided a method to stably label the oxidation state to identify reversible modifications, *S*-Glutathionylation, *S*-nitrosylation and sulfenic acid [15]. Initially, all free thiols undergo alkylation using an alkylating agent e.g. maleimide in the presence of SDS and assisted by heating. This is a critical step for stabilizing the oxidation state and therefore it is best conducted during lysing. Next, any free alkylation agent is removed by a desalting column. The reversible Ox-PTM can be selectively reduced permitting the free thiol to be labelled with a biotin-conjugated alkylating agent such as biotin-HPDP. After termination of the reaction by desalting, biotinylated proteins are purified with streptavidin-agarose beads. Subsequently, the proteins labelled by biotin-HPDP are eluted and can be detected by Western blotting and avidin-based detection protocols [16]. The choice of reducing agent permits the identification of different modifications. For example ascorbate selectively reduces *S*-nitrosylation, sodium arsenite is used to detect sulfenic acid and recombinant-Glrx to identify *S*-glutathionylation.

Glutaredoxin

Glutaredoxins are small (12kd) enzymes belonging to the thioredoxin superfamily of oxidoreductases also known as thioltransferases [17]. Glrx works in conjunction with GSH as the co-substrate to specifically reduce protein-SSG mixed disulfides catalyzing de-glutathionylation. Glrx contain a conserved active site motif, Cys-X-X-Cys [18]. It is the pKa of the N-terminal cysteine in the active site that is of critical importance for Glrx ability to catalyze the thiol-disulfide exchange. This

cysteine has a lower pKa than free cysteine, thus reverses S-glutathionylation via a monothiol mechanism first forming a covalent enzyme intermediate Glrx-SSG which results in release of the reduced protein-SH [18]. Subsequently the Glrx-SSG intermediate undergoes reduction by GSH to produce glutathione disulfide (GSSG) as the secondary product (Figure 2). There are two forms of Glrx in mammals, Glrx-1 and Glrx-2. Glrx-1 is mainly localized in the cytosol, however some reports suggest it may also reside in the nucleus[19]. Glrx-2 is predominantly localized to the mitochondria and also in the nucleus. Human Glrx2 exhibits only 34–36% identity with Glrx-1 and exerts less than 10% of the activity of Glrx-1 [20].

S-glutathionylation affects crucial pathways including cytoskeletal dynamics, protein folding, calcium handling (SERCA) and transcription factors (NF-κB), resulting in structural and functional changes[17]. Key proteins in important signaling pathways like phophatases are also affected by *S*-glutathionylation [21]. Most PTMs are known to induce either activation or inactivation of proteins, in contrast, S-glutathionylation can result in either functional inhibition such as eNOS and NF-κB[22–24] or activation, e.g. SERCA [25,26], depending on the protein and reactive cysteine. Regulation and the role of endogenous Glrx *in vivo* are not well known. Glrx expression is increased in atherosclerotic human coronary artery [27], allergic mouse airway [28], and diabetic rat retina [29] and in type 2 diabetic patients [30], suggesting that up-regulation of Glrx is associated with inflammation and oxidative stress.

Glutathionylation in NF-κB pathway

S-glutathionylation has the potential to negatively regulate the NF-κB pathway at multiple sites, and Glrx activates NF-κB by reversing the modification. The upstream inhibitory kappa kinase (IKK)-β which phosphorylates IκBα causing nuclear translocation of p65 (RelA) is known to be inhibited by *S*-glutathionylation. In alveolar macrophages IKK*β* is glutathionylated at Cys179 by H_2O_2 rendering IKK*β* inactivation, [22] subsequently preventing IκBα degradation and NF-κB translocation. In pancreatic cancer cells hypoxia induced *S*-glutathionylation of p65 inhibiting p65-DNA binding and transcription [31]. Another site of regulation lies at Cys62 of p50, similar to *S*-glutathionylation of p65 this prevents DNA binding of p50 [24]. Glrx reversal of *S*-glutathionylation provides a mechanistic switch to enhance transcription of NF-κB-dependent genes. Thus, Glrx overexpression prolongs NF-κB activation, while Glrx promoter contains two putative NF-κB binding sites and Glrx expression is positively regulated by NF-κB activation[32].

Wnt5a- sFlt-1 signaling

The Wnt protein family is a large group of secreted cysteine-rich glycoproteins, named from the convergence of nomenclature of genes discovered in Drosophila (Wingless) and mammals (Int-1). Signaling by the Wnt family is highly conserved, regulating an extensive array of pathways from embryogenesis to tumorigenesis [33]. Canonical Wnt signaling involves the stabilization and nuclear translocation

of β-catenin upon Wnt ligand binding to the seven-membrane domain receptor Frizzled complexed with Lrp5 [33]. β-catenin-independent signaling, termed non-canonical signaling has been so far described with the following ligands Wnt4, Wnt5a and Wnt11. Non-canonical signaling is mediated via nuclear factor of activated T-cells (NFAT) or Wnt co-receptors Kny, Ror2 and Ryk [33].
A set of studies from Stefatar *et al* reported that Wnt5a, a non-canonical ligand, plays a pivotal role in macrophage regulation of vascular branching during development [34]. In the deep layers of the retina an increase in post-natal angiogenesis was observed in mice lacking Wnt ligand transporter Wntless in myeloid cells. In the deep retinal layer myeloid and EC are in close contact at the sites of vessel branching and it was proven that this interaction allows myeloid cells to specifically control vessels density through Wnt5a regulation of sFlt-1 [34]. The anti-angiogenic receptor sFlt-1 is the soluble isoform of Flt-1 also known as vascular endothelial growth factor receptor 1 (VEGFR1), and sFlt-1 sequesters vascular endothelial growth (VEGF) as it has a higher affinity than the pro-angiogenic receptor, vascular endothelial growth factor receptor 2 (VEGFR2). Adenoviral sFlt-1 gene transfer inhibits ischemic limb revascularization [35]. Also, sFlt-1 is elevated after HLI in diabetic mice which showed impaired revascularization [36].

VEGF signaling and Flt

VEGF is the major factor to stimulate arteriogenesis and angiogenesis. VEGF binds VEGFR2 (Kdr/Flk1) resulting in receptor dimerization and phosphorylation of tyrosine residues. VEGFR2 is the main transducer of VEGF endothelial cell proliferation, migration, and network formation. However, VEGF binds to VEGFR1 (Flt-1) with higher affinity than VEGFR2. Flt-1 gene encodes VEGFR1 protein which contains an extracellular ligand-binding region, a transmembrane segment, and cytoplasmic tyrosine kinase domain. This full-length protein is referred to as the membrane-bound Flt-1 (mFlt). Alternative splicing in intron-13 generates a short isoform which contains only shorter extracellular ligand-binding receptor. This short isoform, soluble protein (sFlt-1) still binds to VEGF but is without kinase activity. Flt-1 can function as a negative regulator for VEGFR2 since a full-length membrane-tethered Flt-1 (mFlt) transduces a weaker signal than VEGFR2, and sFlt-1 can capture the VEGF ligand as a decoy to prevent its binding to VEGFR2 [37]. Elevated sFlt-1 causes pre-eclampsia [38], peripartum cardiomyopathy [39], and impaired ischemic limb vascularization [35].

Up-regulation of Glutaredoxin inhibits neovascularization

Adenoviral overexpression of Glrx in human aortic endothelial cells inhibited VEGF- and nitric oxide (NO)-induced network formation *in vitro* [40]*.* We investigated this finding *in vivo* using a mouse which overexpressed Glrx driven by the β-actin promoter. Up-regulation of Glrx resulted in diminished ischemic limb revascularization in a murine model of hind limb ischemia (HLI) [41]. Furthermore, both treadmill exercise function and capillary density in the gastrocnemius muscle post-HLI were significantly lower than observed in the wild-type littermate controls. EC function was measured in microvascular

endothelial cells selectively ($CD31^+$) isolated from Glrx TG mice [41]. Similar to previous observations in adenoviral Glrx overexpression HAECs, ECs isolated from GlrxTG mice had lower capability of VEGF-induced network formation, migration, and proliferation [41].

Glutaredoxin up-regulates Wnt5a-sFlt-1 in endothelial cells

Interestingly, we found Wnt5a expression to be significantly higher in ischemic muscle and ECs from Glrx TG compared to WT control [41]. Likewise, sFlt-1 levels in plasma from Glrx TG mice after HLI and in the media of Glrx TG ECs were elevated. We revealed that Wnt5a-induced sFlt-1 was also evident in ECs, as recombinant Wnt5a treatment on human microvascular ECs induced sFlt-1, inhibiting network formation [41]. A pharmacological mimetic, Box 5, which competitively inhibits Wnt5a, prevented adenoviral Glrx-dependent increase of sFlt-1 suggesting Wnt5a regulated sFlt-1 in Glrx TG ECs.

Furthermore, knockdown of sFlt-1 restored VEGF-induced migration and network formation in ECs overexpressing Glrx, confirming that enhanced sFlt-1 levels were contributing to Glrx-induced anti-angiogenic effects of EC, and Wnt5a was regulating sFlt-1 expression [41].

Increase in Wnt5a-sFlt-1 expression is p65-dependent

Glrx enhancement of NF-κB activity has been well described[42]. Of importance, Wnt5a promoter B has a NF-κB binding site [43], conversely Wnt5a is able to enhance NF-κB activity suggesting the presence of a positive-feedback loop [44]. In hepatocytes, celecoxib (non-steroidal anti-inflammatory drug) suppressed Wnt5a expression by lowering NF-κB activity [45]. As predicted Glrx TG ECs indeed had the greater potential to enhance NF-κB activity in a luciferase reporter assay[41]. The biotin switch assay as mentioned previously was used to detect glutationylated proteins. Ischemia-induced p65–*S*-glutathionylation in the muscle[41], but the ischemic muscle from Glrx TG mice had significantly less p65-*S*-glutationylation. In human ECs overexpressing Glrx *S*-glutathionylation of p65 was completely abolished compared with ECs treated with the control adenovirus, AdLacZ. Exposing the ECs to hypoxic conditions could not induce p65-*S*-glutationylation in adGlrx overexpressing cells[41]. Interestingly, inhibition of NF-κB can increase tumor vascularization [46], which would be consistent with the current hypothesis that Glrx-mediated activation of NF-κB results in diminished angiogenesis via upregulation of Wnt5a and sFlt-1 (Figure 3).

Summary

Enhanced expression of Glrx has been reported in various pathologies that are associated with oxidative stress, including coronary artery disease and atherosclerotisis [47], allergic airway disease [28], and diabetic retinopathy [48]. Ischemic limb neovascularization was inhibited in the mice overexpressing Glrx in association with sFlt-1 induction. Diabetic patients have higher plasma Glrx levels [30] and a high prevalence for peripheral artery disease. In agreement, the

impaired ischemic limb vascularization correlated with a disproportionate increase in sFlt-1 levels in a diabetic animal model [36]. It suggests that during ischemia *S*-glutathionylation is required to conduct angiogenic signaling, and that enhanced Glrx may contribute to the pathology of impairmed neo-vascularization. . This pathway described for endothelial cells may also occur in other cell types, as Glrx is ubiquitously expressed. Cellular reduction systems are normally regarded to be cytoprotective. Our work, however, suggests that enhancing the Glrx-dependent anti-oxidant system does not promote beneficial ischemic limb neovascularization.

Abbreviations

Glrx: glutaredoxin-1, EC: endothelial cell(s), HLI: hind limb ischemia
sFlt-1: soluble fms-like tyrosine kinase-1, VEGF receptor-1
Ror2: receptor tyrosine kinase-like orphan receptor 2
Wnt5a: wingless-type MMTV integration site family, member 5A
TG: transgenic, VEGFR: VEGF receptor
HPDP: N-[6-(biotinamido) hexyl]-3′-(2′-pyridyldithio) propionamide
NF-κB: nuclear factor of κappa B

Acknowledgements

CE Murdoch is funded by Framework 7 Marie Skłodowska-Curie International Incoming Fellowship [PIIF-GA-2013-626633] at Aston Medical School, Aston University, UK. We thank Richard Cohen at Boston University for the invigorating intellectual environment where these studies were conducted. Work discussed in this article was also supported by National Institutes of Health Grants R37 HL104017, and NHLBI contract number HHSN268201000031C.

References

[1] Murdoch CE, Chaubey S, Zeng L, Yu B, Ivetic A, Walker SJ, et al. (2014) Endothelial NADPH oxidase-2 promotes interstitial cardiac fibrosis and diastolic dysfunction through pro-inflammatory effects and endothelial-mesenchymal transition. J. Am. Coll. Cardiol. **63**.

[2] Murdoch CE, Alom-Ruiz SP, Wang M, Zhang M, Walker S, Yu B, et al. (2011) Role of endothelial Nox2 NADPH oxidase in angiotensin II-induced hypertension and vasomotor dysfunction. Basic Res. Cardiol. **106**, 527–38.

[3] Zimmet JM, Hare JM. (2006) Nitroso-redox interactions in the cardiovascular system. Circulation. **114**, 1531–44.

[4] Burgoyne JR, Mongue-Din H, Eaton P, Shah AM. (2012) Redox signaling in cardiac physiology and pathology. Circ. Res. **111**, 1091–106.

[5] Ray R, Murdoch CE, Wang M, Santos CX, Zhang M, Alom-Ruiz S, et al. (2011) Endothelial Nox4 NADPH oxidase enhances vasodilatation and reduces blood pressure in vivo. Arterioscler. Thromb. Vasc. Biol. **31**, 1368–76.

[6] Holmström KM, Finkel T. (2014) Cellular mechanisms and physiological consequences of redox-dependent signalling. Nat. Rev. Mol. Cell Biol. **15**, 411–21.

[7] Murdoch CE, Zhang M, Cave AC, Shah AM. (2006) NADPH oxidase-dependent redox signalling in cardiac hypertrophy, remodelling and failure. Cardiovasc. Res. **71**, 208–15.

[8] Pimentel D, Haeussler DJ, Matsui R, Burgoyne JR, Cohen RA, Bachschmid MM. (2012) Regulation of cell physiology and pathology by protein S-glutathionylation: lessons learned from the cardiovascular system. Antioxid. Redox Signal. **16**, 524–42.

[9] Murphy E, Kohr M, Sun J, Nguyen T, Steenbergen C. (2012) S-nitrosylation: a radical way to protect the heart. J. Mol. Cell. Cardiol. **52**, 568–77.

[10] Chung HS, Wang S-B, Venkatraman V, Murray CI, Van Eyk JE. (2013) Cysteine oxidative posttranslational modifications: emerging regulation in the cardiovascular system. Circ. Res. **112**, 382–92.

[11] Dulce RA, Schulman IH, Hare JM. (2011) S-glutathionylation: a redox-sensitive switch participating in nitroso-redox balance. Circ. Res. **108**, 531–3.

[12] Dandrea T, Bajak E, Wärngård L, Cotgreave I a. (2002) Protein S-glutathionylation correlates to selective stress gene expression and cytoprotection. Arch. Biochem. Biophys. **406**, 241–52.

[13] Kozarova A, Sliskovic I, Mutus B, Simon ES, Andrews PC, Vacratsis PO. (2007) Identification of redox sensitive thiols of protein disulfide isomerase using isotope coded affinity technology and mass spectrometry. J. Am. Soc. Mass Spectrom. **18**, 260–9.

[14] Foloppe N, Nilsson L. (2004) The glutaredoxin -C-P-Y-C- motif: influence of peripheral residues. Structure. **12**, 289–300.

[15] Jaffrey SR, Snyder SH. (2001) The biotin switch method for the detection of S-nitrosylated proteins. Sci. STKE. **2001**, pl1.

[16] Burgoyne JR, Eaton P. (2011) Contemporary techniques for detecting and identifying proteins susceptible to reversible thiol oxidation. Biochem. Soc. Trans. **39**, 1260–7.

[17] Mieyal JJ, Gallogly MM, Qanungo S, Sabens EA, Shelton MD. (2008) Molecular mechanisms and clinical implications of reversible protein S-glutathionylation. Antioxid. Redox Signal. **10**, 1941–88.

[18] Foloppe N, Vlamis-Gardikas A, Nilsson L. (2012) The -Cys-X1-X2-Cys- motif of reduced glutaredoxins adopts a consensus structure that explains the low pK(a) of its catalytic cysteine. Biochemistry. **51**, 8189–207.

[19] Fernandes AP, Holmgren A. (2004) Glutaredoxins: Glutathione-dependent redox enzymes with functions far beyond a simple thioredoxin backup system. Antioxid. Redox Signal. **6**, 63–74.

[20] Lundberg M, Johansson C, Chandra J, Enoksson M, Jacobsson G, Ljung J, et al. (2001) Cloning and expression of a novel human glutaredoxin (Grx2) with mitochondrial and nuclear isoforms. J. Biol. Chem. **276**, 26269–75.

[21] Barrett WC, DeGnore JP, Keng YF, Zhang ZY, Yim MB, Chock PB. (1999) Roles of superoxide radical anion in signal transduction mediated by reversible regulation of protein-tyrosine phosphatase 1B. J. Biol. Chem. **274**, 34543–6.

[22] Reynaert NL, van der Vliet A, Guala AS, McGovern T, Hristova M, Pantano C, et al. (2006) Dynamic redox control of NF-kappaB through glutaredoxin-regulated S-glutathionylation of inhibitory kappaB kinase beta. Proc. Natl. Acad. Sci. U. S. A. **103**, 13086–91.

[23] Kambe T, Song T, Takata T, Hatano N, Miyamoto Y, Nozaki N, et al. (2010) Inactivation of Ca2+/calmodulin-dependent protein kinase I by S-glutathionylation of the active-site cysteine residue. FEBS Lett. **584**, 2478–84.

[24] Pineda-Molina E, Klatt P, Vázquez J, Marina a, García de Lacoba M, Pérez-Sala D, et al. (2001) Glutathionylation of the p50 subunit of NF-kappaB: a mechanism for redox-induced inhibition of DNA binding. Biochemistry. **40**, 14134–42.

[25] Adachi T, Weisbrod RM, Pimentel DR, Ying J, Sharov VS, Schöneich C, et al. (2004) S-Glutathiolation by peroxynitrite activates SERCA during arterial relaxation by nitric oxide. Nat. Med. **10**, 1200–7.

[26] Hawkins BJ, Irrinki KM, Mallilankaraman K, Lien Y-C, Wang Y, Bhanumathy CD, et al. (2010) S-glutathionylation activates STIM1 and alters mitochondrial homeostasis. J. Cell Biol. **190**, 391–405.

[27] Okuda M, Inoue N, Azumi H, Seno T, Sumi Y, Hirata Ki, et al. (2001) Expression of glutaredoxin in human coronary arteries: its potential role in antioxidant protection against atherosclerosis. Arterioscler. Thromb. Vasc. Biol. **21**, 1483–7.

[28] Reynaert NL, Wouters EFM, Janssen-Heininger YMW. (2007) Modulation of glutaredoxin-1 expression in a mouse model of allergic airway disease. Am. J. Respir. Cell Mol. Biol. **36**, 147–51.

[29] Shelton MD, Kern TS, Mieyal JJ. (2007) Glutaredoxin regulates nuclear factor kappa-B and intercellular adhesion molecule in Müller cells: model of diabetic retinopathy. J. Biol. Chem. **282**, 12467–74.

[30] Du Y, Zhang H, Montano S, Hegestam J, Ekberg NR, Holmgren A, et al. (2014) Plasma glutaredoxin activity in healthy subjects and patients with abnormal glucose levels or overt type 2 diabetes. Acta Diabetol. **51**, 225–232.

[31] Qanungo S, Starke DW, Pai H V, Mieyal JJ, Nieminen A-L. (2007) Glutathione supplementation potentiates hypoxic apoptosis by S-glutathionylation of p65-NFkappaB. J. Biol. Chem. **282**, 18427–36.

[32] Aesif SW, Kuipers I, van der Velden J, Tully JE, Guala AS, Anathy V, et al. (2011) Activation of the glutaredoxin-1 gene by nuclear factor κB enhances signaling. Free Radic. Biol. Med. **51**, 1249–57.

[33] Kikuchi A, Yamamoto H, Sato A, Matsumoto S. (2012) Wnt5a: its signalling, functions and implication in diseases. Acta Physiol. (Oxf). **204**, 17–33.

[34] Stefater JA, Lewkowich I, Rao S, Mariggi G. (2011) Regulation of angiogenesis by a non-canonical Wnt-Flt1 pathway in myeloid cells. Nature. **474**, 511–515.

[35] Jacobi J, Tam BYY, Wu G, Hoffman J, Cooke JP, Kuo CJ. (2004) Adenoviral gene transfer with soluble vascular endothelial growth factor receptors impairs angiogenesis and perfusion in a murine model of hindlimb ischemia. Circulation. **110**, 2424–9.

[36] Hazarika S, Dokun AO, Li Y, Popel AS, Kontos CD, Annex BH. (2007) Impaired angiogenesis after hindlimb ischemia in type 2 diabetes mellitus: differential regulation of vascular endothelial growth factor receptor 1 and soluble vascular endothelial growth factor receptor 1. Circ. Res. **101**, 948–56.

[37] Kendall RL, Thomas KA. (1993) Inhibition of vascular endothelial cell growth factor activity by an endogenously encoded soluble receptor. Proc. Natl. Acad. Sci. U. S. A. **90**, 10705–9.

[38] Ahmad S, Ahmed A. (2004) Elevated placental soluble vascular endothelial growth factor receptor-1 inhibits angiogenesis in preeclampsia. Circ. Res. **95**, 884–91.

[39] Patten IS, Rana S, Shahul S, Rowe GC, Jang C, Liu L, et al. (2012) Cardiac angiogenic imbalance leads to peripartum cardiomyopathy. Nature. **485**, 333–8.

[40] Evangelista AM, Thompson MD, Weisbrod RM, Pimental DR, Tong X, Bolotina VM, et al. (2012) Redox Regulation of SERCA2 Is Required for Vascular Endothelial Growth Factor-Induced Signaling and Endothelial Cell Migration. Antioxid. Redox Signal. **17**, 1099–108.

[41] Murdoch CE, Shuler M, Haeussler DJ, Kikuchi R, Bearelly P, Han J, et al. (2014) Glutaredoxin-1 up-regulation induces soluble vascular endothelial growth factor receptor 1, attenuating post-ischemia limb revascularization. J Biol Chem.

[42] Lim SY, Raftery MJ, Goyette J, Geczy CL. (2010) S-Glutathionylation Regulates Inflammatory Activities. J. Biol. Chem. **285**, 14377–14388.

[43] Katoh M, Katoh M. (2009) Transcriptional mechanisms of WNT5A based on NF- κ B , Hedgehog , TGFß , and Notch signaling cascades. Internatinal J. Mol. Med. **23**, 763–769.

[44] Kim J, Kim J, Kim DW, Ha Y, Ihm MH, Kim H, et al. (2010) Wnt5a induces endothelial inflammation via beta-catenin-independent signaling. J. Immunol. **185**, 1274–82.

[45] Tian F, Zhang YJ, Li Y, Xie Y. (2014) Celecoxib ameliorates non-alcoholic steatohepatitis in type 2 diabetic rats via suppression of the non-canonical Wnt signaling pathway expression. PLoS One. **9**, e83819.

[46] Kisseleva T, Song L, Vorontchikhina M, Feirt N, Kitajewski J, Schindler C. (2006) NF-κB regulation of endothelial cell function during LPS-induced toxemia and cancer. J. Clin. Invest. **116**, 2955–2963.

[47] Okuda M, Inoue N, Azumi H, Seno T. (2001) Expression of glutaredoxin in human coronary arteries its potential role in antioxidant protection against atherosclerosis. Arterioscler. Thromb. Vasc. Biol. Vasc. Biol. **21**, 1483–1487.

[48] Shelton MD, Distler AM, Kern TS, Mieyal JJ. (2009) Glutaredoxin regulates autocrine and paracrine proinflammatory responses in retinal glial (muller) cells. J. Biol. Chem. **284**, 4760–6.

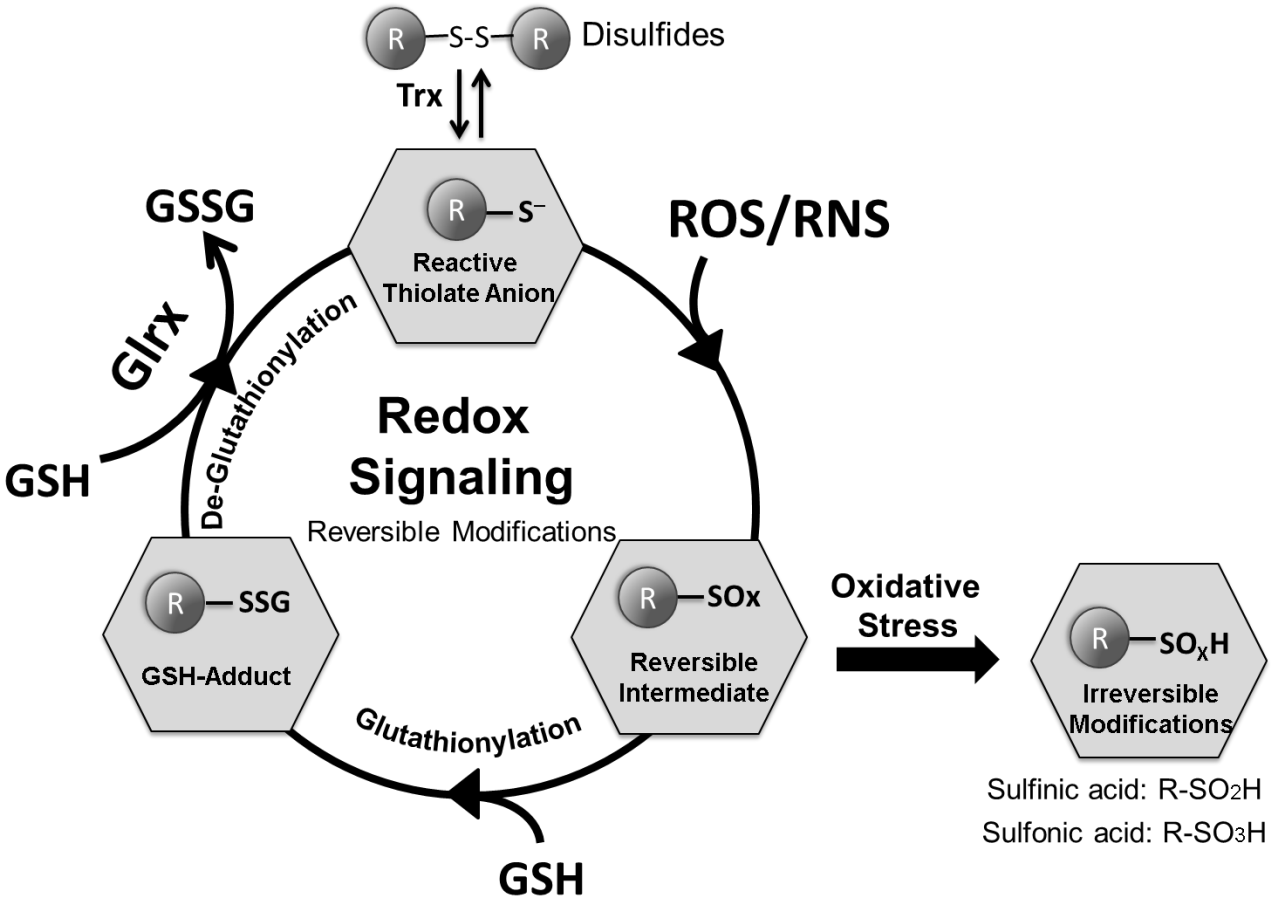

Figure 1: Reactive protein thiolates undergo reversible or irreversible oxidation. Reactive oxygen species/ reactive nitrogen species (ROS/RNS) generate reversible intermediates including *S*-nitrosothiol (R-SNO) and sulfenic acid (R-SOH), which may become *S*-glutathionylated protein (R-SSG). R-SOH can be further oxidized to generate irreversible modifications such as sulfonic acid ($R\text{-}SO_3H$). ROS, reactive oxygen species; RNS, reactive nitrogen species; Glrx, Glutaredoxin; GSH, glutathione; Trx, thioredoxin.

Figure 2: Glutaredoxin system. R-SSG, S-glutathionylated protein; GR, glutathione reductase; Glrx, glutaredoxin-1, catalyze removal of GSH adducts in the presence of GSH and NADPH.

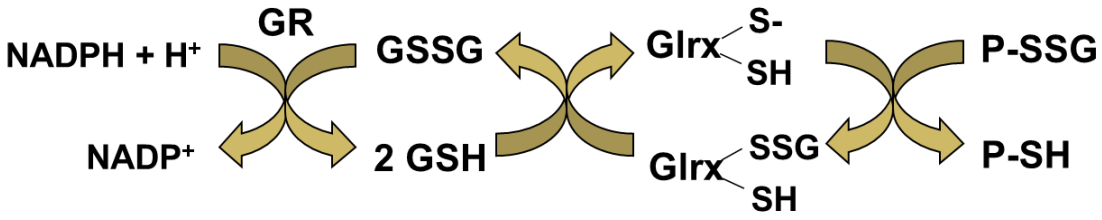

Figure 3: Enhanced endothelial Glrx increases NF-κB-dependent Wnt5a, upregulating the anti-angiogenic receptor sFlt-1. Glrx, Glutaredoxin; VEGF, vascular endothelial growth factor; sFlt-1, soluble fms-like tyrosine kinase-1 (VEGFR1); Wnt5a, wningless-type MMTC integration site family, member 5A; ROR2**,** receptor tyrosine kinase-like orphan receptor 2; –SSG, *S*-glutathionylation.