Thioredoxin and glutaredoxin-mediated redox regulation of ribonucleotide reductase

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Abstract

Ribonucleotide reductase (RNR), the rate-limiting enzyme in DNA synthesis, catalyzes reduction of the different ribonucleotides to their corresponding deoxyribonucleotides. The crucial role of RNR in DNA synthesis has made it an important target for the development of antiviral and anticancer drugs. Taking account of the recent developments in this field of research, this review focuses on the role of thioredoxin and glutaredoxin systems in the redox reactions of the RNR catalysis.

Key words: Ribonucleotide reductase; Thioredoxin; Glutaredoxin; DNA synthesis; Thiol disulfides; Replication

Core tip: Thioredoxin and glutaredoxin-mediated redox regulations of ribonucleotide reductase (RNR) catalysis play a vital role as the RNR catalysis involves different redox active thiol functions, thiyl radicals and thiol proteins. The in depth knowledge of the whole redox catalysis will contribute significantly to designing and developing new RNR inhibitors for improved cancer chemotherapy, antibiotic development and antiviral treatments.

INTRODUCTION

Ribonucleotide reductase (RNR) catalyzes the rate limiting step of the DNA synthesis where the reduction of ribonucleotides (NTPs) results in the formation of corresponding deoxyribonucleotides (dNTPs)[1-4]. The RNR catalysis involves protein free radicals, redox-active thiols and proteins of the thioredoxin (Trx) superfamily. In the RNR complex, the R1 subunit contains the active site, allosteric sites and redox active thiols/disulfides required for the RNR catalysis; while the R2 subunit provides a dinuclear metal cluster and a tyrosyl free radical essential for the catalytic cycle. The RNR activity can be regulated by expression of different subunits, subcellular localization, post-translational modifications and allosteric regulation involving both activity and substrate specificity[3-8]. The DNA replication is coordinated with the cell growth by different regulatory mechanisms. Development of malignancy and cancer are found to be associated with an increased expression and activity of RNR. In cells, an imbalance in the levels of dNTPs will cause mutagenesis and carcinogenesis. On the other hand, blockage of RNR activity can inhibit DNA synthesis and repair which results in apoptosis. In recent years, several RNR inhibitors have entered clinical trials. Recent developments in the field will provide a new basis for the discovery of more
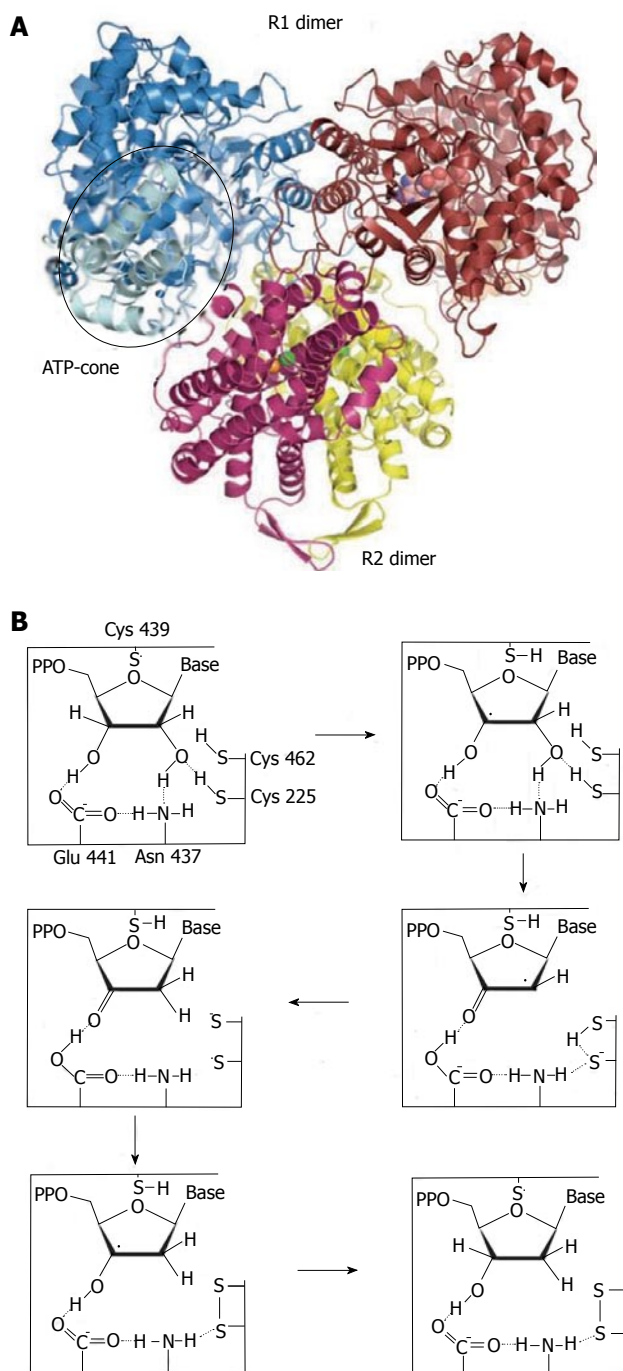
A

R1 dimer

ATP-cone

R2 dimer

B

Figure 1 Crystal structure of Class I aerobic ribonucleotide reductase complex (A), and proposed reaction mechanism of ribonucleotide reductase catalysis (B). A: This is based on the crystal structures of the R1 and R2 proteins (Protein Data Bank ID: 1RLR and 1RIB). The figure shows the presence of substrate in R1 subunit and dinuclear iron center in R2 subunit. The ribonucleotide reductase complex (RNR) complex is a tetramer with the dimer of R1 subunit and the dimer of R2 subunit. The allosteric regulatory domain of R1 subunit (ATP-cone) binds either ATP or dATP to regulate the enzymatic activity (adapted from Logan *et al*[9]); B: The figure describes the reduction of nucleoside diphosphate (NDP) to deoxyribonucleoside diphosphates (dNDP) by class I RNR (*E. coli*). The reduction is initiated by a thiyl radical (Cys 439) by abstracting the 3'-hydrogen from the NDP. A water molecule is lost and the two cysteines (Cys 225 and Cys 462) then deliver the required reducing equivalents, generating a 3'-ketodeoxynucleotide which is subsequently reduced to give dNDP (adapted from Holmgren *et al*[4]).

effective RNR inhibitors for cancer therapy. Taking account of the recent progress in this field of research, this

review focuses on the role of Trx and glutaredoxin (Grx) systems in the redox regulations of the RNR catalysis.

RNR: CLASSIFICATION AND CATALYSIS

Based on the pathways of radical initiation and requirements of metal cofactors, the RNRs have been divided in to three classes[2-5]. The active site of all three classes of RNR has a very similar structure (Figure 1)[2-5,9]. A conserved cysteine residue plays the vital role for the generation of a thiyl radical in all the classes of RNR (Figures 1 and 2). The reaction mechanisms of different classes of RNR are similar due to the structural similarities of the catalytic domains. The RNR catalysis starts with the generation of a thiyl radical close to the bound substrate[2-5]. Then the abstraction of hydrogen from the C3' of the ribose ring and generation of a substrate radical occur. During the RNR catalysis, a cysteinyl radical, required for the abstraction of hydrogen at the C3' ribose substrate, is derived from a tyrosyl radical for class I or cobalamin cofactor for class II or a glycyl radical for class III. For class I and II enzymes, electrons required for the reduction of the ribonucleotides aare provided by reduced nicotinamide adenine dinucleotide phosphate (NADPH) through Trx or Grx systems. However, for class III enzymes, the electrons are supplied by formate.

The class I RNR occurs in eukaryotes, eubacteria, bacteriophages and viruses. The complex, in its simple form, is a tetramer with the dimer of larger subunit (R1) and the dimer of R2 subunit (Figures 1 and 2)[2-5,9]. Oxygen is required for the generation of a tyrosyl radical (Tyr 122) in the R2 subunit. As described above, during catalysis, the radical is continuously transferred to a cysteine (Cys 439) residue of the R1 subunit and generates a thiyl radical to activate the substrate. The R1 subunit carries the catalytic site, allosteric effector binding sites and redox-active thiol groups required for the catalysis. p53R2 is an additional mammalian RNR protein which functions as a catalytic partner of the R1 subunit[10-12]. The expression of the p53R2 subunit is induced by DNA damage which is mediated by the tumor suppressor protein p53. Both R2 and p53R2 subunits use a diferric iron center generating a tyrosyl free radical required for the RNR catalysis. The R1-p53R2 complex is suggested to be required for basal DNA repair and the R1–R2 complex is suggested to be associated with DNA replication[11]. Moreover, the R1-p53R2 complex has been found to play a significant role in dNTP supply for mitochondrial DNA synthesis.

For class II RNR (archaebacteria, eubacteria), a cofactor (5'-deoxyadenosylcobalamin) replaces the presence of a separate subunit for storage of radicals[2-5]. The cleavage of 5'-deoxyadenosylcobalamin generates a deoxyadenosyl radical which abstracts hydrogen from the active site cysteine residue. Trx system can reduce the C-terminal pair of redox-active cysteines which, in turn, can reduce the active site to continue the RNR catalysis. For anaerobic class III RNR (archaebacteria, eubacteria, bacteriophages), a glycyl radical is generated by the action of activase, S-adenosylmethionine and a reducing
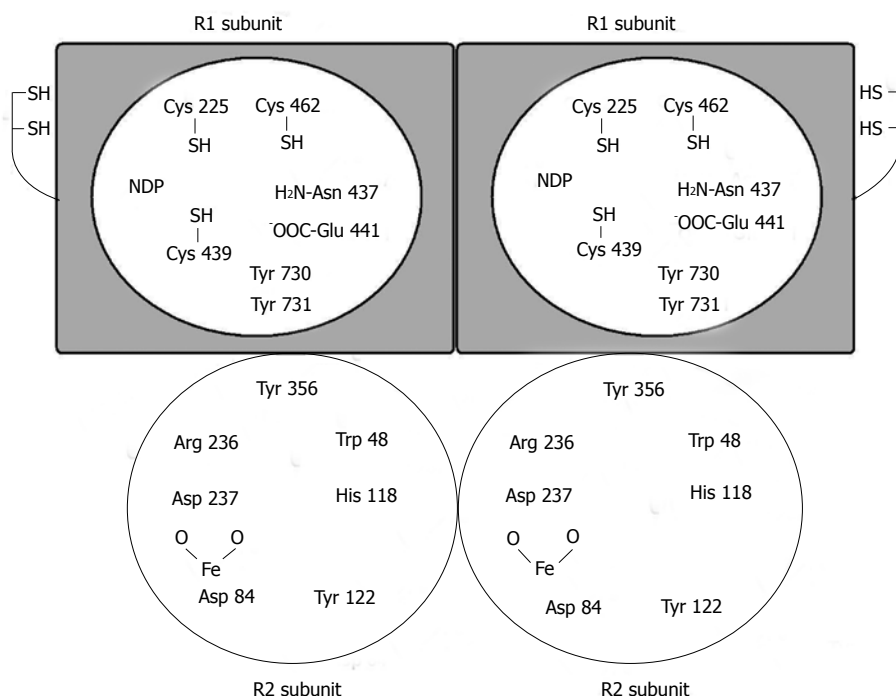
Figure 2 Subunit organization of ribonucleotide reductase complex. Amino acids are shown with *E. coli* numbering which are crucial for the radical transfer and ribonucleotide reductase (RNR) catalysis. The R2 subunit contains the iron-oxygen cluster (Fe-O-Fe) which reacts with dioxygen to generate a stable tyrosyl radical in Tyr 122 required for the RNR catalysis. The radical transfer pathway from Tyr 122 to the active-site Cys 439 in R1 subunit involves the network of Asp 84, His 118, Asp 237, Trp 48, Tyr 356 in R2 subunit and Tyr 730, Tyr 731 in R1 subunit[2-5]. The Cys 225, Cys 462, Asn 437 and Glu 441 residues are involved in binding the substrate nucleoside diphosphate (NDP) in R1 subunit. During the catalysis, the disulfide bond between Cys 225 and Cys 462 is reduced by the C-terminal shuttle dithiols[2-5]. The figure is adapted and modified from Holmgren *et al*[4].

system containing flavoredoxin, NADPH and NADPH-flavoredoxin reductase. Then the glycyl radical generates a thiyl radical required for the catalysis.

TRX AND GRX SYSTEM

Trx is a class of 12 kDa ubiquitous redox proteins found primarily in the cytosol[13]. Trxs possess a catalytically active dithiol function in a Cys-Gly-Pro-Cys motif and are present in all organisms. The complete mammalian Trx system comprising Trx, the selenoenzyme thioredoxin reductase (TrxR) and NADPH also plays a crucial role in redox signaling and thiol homeostasis of cells. Cytosolic Trx1 and mitochondrial Trx2 regulate several metabolic pathways, oxidative/nitrosative stress defence, apoptosis and DNA synthesis[4,13-15]. On the other hand, Grxs are small redox enzymes of approximately 10 kDa and they participate in thiol-disulfide exchange reactions in the presence of glutathione (GSH), glutathione reductase (GR) and NADPH. In the Grx system, Grx is reduced *via* GSH[16]. The glutathione disulfide formed is then reduced by GR and NADPH. Grxs are involved in redox signaling and maintenance of cellular redox environment. Moreover, the maintenance of cytosolic and mitochondrial iron homeostasis have been found to be linked to Grxs[16,17].

Several findings support the redundancy between cytosolic Trx and Grx systems to provide a backup for each other. Yeast and bacterial strains can survive in the absence of either of the disulphide reductase pathways[18].

Moreover, GSH synthesis was found to be essential for mouse development[19], whereas the deletion of Trx gene was reported to be lethal for mouse embryo[20]. Loss of TrxR1 showed no effect on the normal replicative potential[21]. However, the survival of TrxR1-deficient tumor cells was found to be very much dependent on GSH[22]. In a recent study, it has also been shown that the GSH/Grx system can reduce Trx1 in TrxR1-deficient HeLa cells[23].

ROLE OF TRX AND GRX AS EXTERNAL ELECTRON DONORS FOR RNR

Trxs and Grxs belong to related families of low molecular weight redox enzymes catalyzing thiol-disulfide exchange reactions with catalytically active cysteine thiols in a CXXC active site[13,16]. For class I and II RNR enzymes, the electrons are supplied by NADPH through Trx or Grx systems[2-5]. Reduction of ribonucleotide in the RNR catalysis involves the formation of a disulfide in the active site of R1 subunit. Structural studies with *E. coli* RNR show that the active site cleft of the R1 subunit is not very wide to permit the direct reduction by the external redoxin system(s)[3,24]. However, the reduction of active site disulfide is performed by a pair of shuttle cysteine residues in the C-terminal mobile tail of R1 subunit (Figure 3)[4,25,26]. The C-terminal shuttle dithiols of *E. coli* R1 subunit has the CXXXXC sequence; whereas yeast and mammalian R1 has a CXXC sequence. *In vitro*
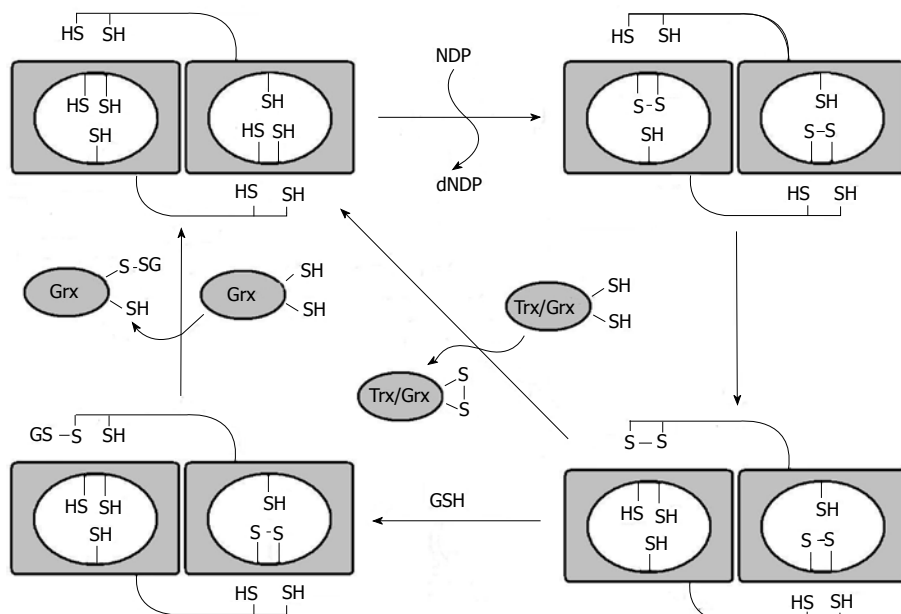
Figure 3 The mechanistic model for the role of thioredoxins and glutaredoxins for the ribonucleotide reductase catalysis. After the completion of one turnover cycle of ribonucleotide reductase (RNR) catalysis, a disulfide bond is formed between the conserved cysteine pair at the active site (shown in the circle). Shuttle dithiol function present at the C-terminal CXXC motif of the neighboring subunit reduces the disulfide bond through disulfide-exchange. Then, the resulting disulfide bond at the C-terminal tail is reduced by the thioredoxin/glutaredoxin (Trx/Grx) systems resulting in an active R1 to continue the next cycle of RNR catalysis. The Grx system can also reduce the C-terminal thiols by the glutathionylation mechanism[4,25,26]. For simplicity, only the reduction of active site of one subunit by the C-terminal shuttle dithiols of the neighboring subunit is shown in the diagram. The figure is adapted and modified from Holmgren *et al*[4].

mutagenesis and kinetic studies support a critical role for the C-terminal cysteine pair of R1 in regeneration of the active site[27,28]. The disulfide exchange reaction results in the formation of a disulfide in the C-terminal tail of R1. Then, the external redoxin systems reduce the disulfide bond to continue the next cycle of RNR catalysis.

Trx and Grx systems were found to act as dithiol electron donors of *E. coli* RNR[29,30]. Recently, Gustafsson *et al*[31] characterized the Trx1 system as the physiologically relevant electron donor for RNR in *Bacillus anthracis*. In *E. coli*, the class I a enzyme requires the dithiol form of at least one of Grx1, Trx1 or Trx2 to be viable[32-34]. The Grx1 system showed 10-fold lower k_m value compared to that of Trx1 system, while both of the redoxins had similar v_{max} (Table 1). This makes Grx1 the most efficient electron donor for the *E. coli* enzyme. However, there is a mechanistic difference between the *E. coli* and the mammalian RNR catalysis involving Trx1 and Grx1 systems as electron donors[26]. Trx1 and Grx1 system showed similar catalytic efficiencies (k_{cat}/k_m) with recombinant mouse RNR complex (Figure 4 and Table 1). In the presence of 4 mmol/L GSH, the Grx1 system showed a higher affinity compared to Trx1 and displayed a higher apparent k_{cat}. The RNR activity with the Grx system was found to be very much dependent on the concentrations of GSH. Here, it is noteworthy to mention that mammalian cells have significantly high concentrations of GSH (5-20 mmol/L)[35]. Moreover, the ability of the monothiol mutant of Grx2 to maintain RNR catalysis demonstrates a glutathionylation mechanism for Grx catalysis in contrast to the dithiol mechanism for the Trx system[26]. However, the *E. coli* RNR complex showed

no activity with the monothiol mutant of bacterial Grx1 suggesting the involvement of a dithiol-disulfide mechanism for the catalysis[36].

The advantage of a glutathionylation mechanism may be with the very low levels of R1 involved in the repair and production of dNTPs for mitochondrial DNA synthesis. Trx is present at low levels in many resting postmitotic cells. The sigmoidal curve of Trx activity showed that reduced Trx could not be efficient with a low concentration of R1 in postmitotic cells[26]. The high concentration of GSH[35] would ensure that there is glutathionylated R1 and then monothiol/dithiol Grx should be able to catalyze reduction of the C-terminal disulfide. Several studies reported that the rapidly proliferating cells have increased GSH concentration, while a decrease in GSH concentration limits cell proliferation. GSH acts as a key regulator of cell proliferation and thus the colocalization of GSH with nuclear DNA was observed in proliferating cells[37]. In mammary carcinoma cells, the depletion of glutathione was found to inhibit DNA synthesis[38]. A similar study with 3T3 fibroblast cells showed a significant correlation between progression of cell cycle and the distribution of nuclear GSH[39]. Moreover, accumulation of DNA damage was found in liver, kidney and lung of mice deficient in γ-glutamyl transpeptidase, the enzyme responsible for initiating the catabolism of GSH[40]. In another study, down-regulation of TrxR showed no effects on the dNTP pools in malignant mouse cells[41]. This suggests the role of the GSH/Grx system as an alternative pathway used by the RNR in tumor cells. Moreover, a study in mouse hepatocytes, suggested the importance of a TrxR-independent pathway for the supply of electrons

Table 1 Kinetic parameters of thioredoxin 1, glutaredoxins (1, 2, 2C40S) for reduction of mouse ribonucleotide reductase complex		
Electron donor system	V_{max} (nmol/L per second per/ microgram of R1)	k_m (μmol/L)
Trx1	22.2	1.90
Grx1	2.3	0.18
Grx2	1.5	0.30
Grx2C40S	1.5	0.36

Data adapted from Zahedi Avval *et al*[26]. Trx: Thioredoxin; Grx: Glutaredoxins.

to RNR[21]. These studies clearly show the crucial role of the GSH/Grx system in DNA repair *via* RNR catalysis.

ROLE OF TRX FOR CLASS Ⅲ RNR

The class Ⅲ RNR (present in strict and facultative anaerobes) forms an inactive $\alpha_2\beta_2$ complex in resting state[2-5]. The cysteine residues present in the C-terminus of protein α were found to be responsible for the formation of glycyl radical and to participate in radical transfer reactions during enzyme activation. Under the reducing condition, the small β subunit can activate several α proteins. The Trx system was found to activate the enzyme with the same efficiency as dithiothreitol (DTT)[42]. The data suggests that the Trx system keeps the conserved cysteines of the C-terminus of the α-polypeptide in a reduced form which is required for radical generation. Therefore, Trx acts only for the activation of the class Ⅲ RNR. Later, a structural study of the homologous enzyme from bacteriophage T4 revealed the presence of zinc bound to four conserved cysteine residues[43]. It was also shown that the Trx system or DTT is dispensable for the formation of the glycyl radical with the fully Zn-loaded RNR. The radical transfer from glycine to the active-site cysteine to the substrate is controlled by a crucial hydrogen-bond network. Thus, the suggested role of the Trx system (or DTT) was to facilitate the recognition of the network and allow efficient radical transfer.

CONCLUSION

For several years, the RNR inhibitors have been used to treat cancers and viral infections[6,7,44,45]. Most of the RNR inhibitors are either radical scavengers (hydroxyurea) or metal chelators (triapine) which specifically inactivate the R2 subunit[46,47]. On the other hand, several nucleoside analogs and sulfhydryl group inhibitors (such as cisplatin, caracemide, chlorambucil, *etc*.) are used as R1-specific inhibitors[48-50]. Gene expression silencers and R1-R2 polymerization inhibitors (oligopeptides) have also been used to block RNR activity[51-55]. In recent years, many new strategies have emerged in the designing of subunit-specific and more effective RNR inhibitors. Redox regulation of RNR catalysis plays a vital role as the RNR catalysis involves different redox active thiol functions, thiyl radicals and thiol proteins of the Trx superfamily.
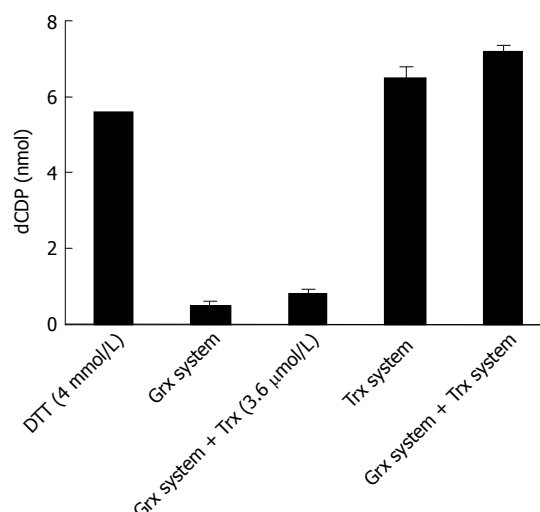

Figure 4 **Activity profile of mouse ribonucleotide reductase in the presence of the thioredoxin and glutaredoxins system.** Mouse R1 (120 μg/mL) and R2 (40 μg/mL) were assayed with dithiothreitol, thioredoxin (Trx) and glutaredoxin (Grx) systems. The Trx system contained 3.6 μmol/L Trx1, NADPH and TrxR. The Grx system contained 1 μmol/L Grx1, 4 mmol/L glutathione , NADPH and glutathione reductase. Combinations of 3.6 μmol/L Trx1 or the whole Trx system with the Grx system were also monitored (data adapted from Zahedi Avval *et al*[26]).

Therefore, further investigations on the Trx/Grx-mediated redox regulation of RNR catalysis will contribute significantly to design and develop new RNR inhibitors for improved cancer chemotherapy and antiviral treatments.

REFERENCES

1   **Holmgren A**, Reichard P, Thelander L. Enzymatic synthesis of deoxyribonucleotides, 8. The effects of ATP and dATP in the CDP reductase system from E. coli. *Proc Natl Acad Sci USA* 1965; **54**: 830-836 [PMID: 5324398 DOI: 10.1073/pnas.54.3.830]

2   **Jordan A**, Reichard P. Ribonucleotide reductases. *Annu Rev Biochem* 1998; **67**: 71-98 [PMID: 9759483 DOI: 10.1146/annurev.biochem.67.1.71]

3   **Nordlund P**, Reichard P. Ribonucleotide reductases. *Annu Rev Biochem* 2006; **75**: 681-706 [PMID: 16756507 DOI: 10.1146/annurev.biochem.75.103004.142443]

4   **Holmgren A**, Sengupta R. The use of thiols by ribonucleotide reductase. *Free Radic Biol Med* 2010; **49**: 1617-1628 [PMID: 20851762 DOI: 10.1016/j.freeradbiomed.2010.09.005]

5   **Kolberg M**, Strand KR, Graff P, Andersson KK. Structure, function, and mechanism of ribonucleotide reductases. *Biochim Biophys Acta* 2004; **1699**: 1-34 [PMID: 15158709]

6   **Kwon NS**, Stuehr DJ, Nathan CF. Inhibition of tumor cell ribonucleotide reductase by macrophage-derived nitric oxide. *J Exp Med* 1991; **174**: 761-767 [PMID: 1717630]

7   **Shao J**, Liu X, Zhu L, Yen Y. Targeting ribonucleotide reductase for cancer therapy. *Expert Opin Ther Targets* 2013; **17**: 1423-1437 [PMID: 24083455 DOI: 10.1517/14728222.2013.840293]

8   **Håkansson P**, Hofer A, Thelander L. Regulation of mammalian ribonucleotide reduction and dNTP pools after DNA damage and in resting cells. *J Biol Chem* 2006; **281**: 7834-7841 [PMID: 16436374]

9   **Logan DT**. Closing the circle on ribonucleotide reductases. *Nat Struct Mol Biol* 2011; **18**: 251-253 [PMID: 21372851 DOI: 10.1038/nsmb0311-251]

10 **Tanaka H**, Arakawa H, Yamaguchi T, Shiraishi K, Fukuda S, Matsui K, Takei Y, Nakamura Y. A ribonucleotide reductase

gene involved in a p53-dependent cell-cycle checkpoint for DNA damage. *Nature* 2000; **404**: 42-49 [PMID: 10716435]

11   **Thelander L**. Ribonucleotide reductase and mitochondrial DNA synthesis. *Nat Genet* 2007; **39**: 703-704 [PMID: 17534360]

12   **Bourdon A**, Minai L, Serre V, Jais JP, Sarzi E, Aubert S, Chrétien D, de Lonlay P, Paquis-Flucklinger V, Arakawa H, Nakamura Y, Munnich A, Rötig A. Mutation of RRM2B, encoding p53-controlled ribonucleotide reductase (p53R2), causes severe mitochondrial DNA depletion. *Nat Genet* 2007; **39**: 776-780 [PMID: 17486094]

13   **Lillig CH**, Holmgren A. Thioredoxin and related molecules--from biology to health and disease. *Antioxid Redox Signal* 2007; **9**: 25-47 [PMID: 17115886]

14   **Sengupta R**, Holmgren A. Thioredoxin and thioredoxin reductase in relation to reversible S-nitrosylation. *Antioxid Redox Signal* 2013; **18**: 259-269 [PMID: 22702224 DOI: 10.1089/ars.2012.4716]

15   **Sengupta R**, Holmgren A. The role of thioredoxin in the regulation of cellular processes by S-nitrosylation. *Biochim Biophys Acta* 2012; **1820**: 689-700 [PMID: 21878369 DOI: 10.1016/j.bbagen.2011.08.012]

16   **Lillig CH**, Berndt C, Holmgren A. Glutaredoxin systems. *Biochim Biophys Acta* 2008; **1780**: 1304-1317 [PMID: 18621099 DOI: 10.1016/j.bbagen.2008.06.003]

17   **Johansson C**, Roos AK, Montano SJ, Sengupta R, Filippakopoulos P, Guo K, von Delft F, Holmgren A, Oppermann U, Kavanagh KL. The crystal structure of human GLRX5: iron-sulfur cluster co-ordination, tetrameric assembly and monomer activity. *Biochem J* 2011; **433**: 303-311 [PMID: 21029046 DOI: 10.1042/BJ20101286]

18   **Trotter EW**, Grant CM. Non-reciprocal regulation of the redox state of the glutathione-glutaredoxin and thioredoxin systems. *EMBO Rep* 2003; **4**: 184-188 [PMID: 12612609]

19   **Shi ZZ**, Osei-Frimpong J, Kala G, Kala SV, Barrios RJ, Habib GM, Lukin DJ, Danney CM, Matzuk MM, Lieberman MW. Glutathione synthesis is essential for mouse development but not for cell growth in culture. *Proc Natl Acad Sci USA* 2000; **97**: 5101-5106 [PMID: 10805773]

20   **Matsui M**, Oshima M, Oshima H, Takaku K, Maruyama T, Yodoi J, Taketo MM. Early embryonic lethality caused by targeted disruption of the mouse thioredoxin gene. *Dev Biol* 1996; **178**: 179-185 [PMID: 8812119]

21   **Rollins MF**, van der Heide DM, Weisend CM, Kundert JA, Comstock KM, Suvorova ES, Capecchi MR, Merrill GF, Schmidt EE. Hepatocytes lacking thioredoxin reductase 1 have normal replicative potential during development and regeneration. *J Cell Sci* 2010; **123**: 2402-2412 [PMID: 20571049 DOI: 10.1242/jcs.068106]

22   **Mandal PK**, Schneider M, Kölle P, Kuhlencordt P, Förster H, Beck H, Bornkamm GW, Conrad M. Loss of thioredoxin reductase 1 renders tumors highly susceptible to pharmacologic glutathione deprivation. *Cancer Res* 2010; **70**: 9505-9514 [PMID: 21045148 DOI: 10.1158/0008-5472.CAN-10-1509]

23   **Du Y**, Zhang H, Lu J, Holmgren A. Glutathione and glutaredoxin act as a backup of human thioredoxin reductase 1 to reduce thioredoxin 1 preventing cell death by aurothioglucose. *J Biol Chem* 2012; **287**: 38210-38219 [PMID: 22977247 DOI: 10.1074/jbc.M112.392225]

24   **Uhlin U**, Eklund H. Structure of ribonucleotide reductase protein R1. *Nature* 1994; **370**: 533-539 [PMID: 8052308]

25   **Zhang Z**, Yang K, Chen CC, Feser J, Huang M. Role of the C terminus of the ribonucleotide reductase large subunit in enzyme regeneration and its inhibition by Sml1. *Proc Natl Acad Sci USA* 2007; **104**: 2217-2222 [PMID: 17277086]

26   **Zahedi Avval F**, Holmgren A. Molecular mechanisms of thioredoxin and glutaredoxin as hydrogen donors for Mammalian s phase ribonucleotide reductase. *J Biol Chem* 2009; **284**: 8233-8240 [PMID: 19176520 DOI: 10.1074/jbc.M809338200]

27   **Aberg A**, Hahne S, Karlsson M, Larsson A, Ormö M, Ahgren A, Sjöberg BM. Evidence for two different classes of redox-active cysteines in ribonucleotide reductase of Escherichia coli. *J Biol Chem* 1989; **264**: 12249-12252 [PMID: 2663852]

28   **Lin AN**, Ashley GW, Stubbe J. Location of the redox-active thiols of ribonucleotide reductase: sequence similarity between the Escherichia coli and Lactobacillus leichmannii enzymes. *Biochemistry* 1987; **26**: 6905-6909 [PMID: 3322391]

29   **Laurent TC**, Moore ec, reichard p. enzymatic synthesis of deoxyribonucleotides. IV. Isolation and characterization of thioredoxin, the hydrogen donor from escherichia coli b. *J Biol Chem* 1964; **239**: 3436-3444 [PMID: 14245400]

30   **Holmgren A**. Hydrogen donor system for Escherichia coli ribonucleoside-diphosphate reductase dependent upon glutathione. *Proc Natl Acad Sci USA* 1976; **73**: 2275-2279 [PMID: 7783]

31   **Gustafsson TN**, Sahlin M, Lu J, Sjöberg BM, Holmgren A. Bacillus anthracis thioredoxin systems, characterization and role as electron donors for ribonucleotide reductase. *J Biol Chem* 2012; **287**: 39686-39697 [PMID: 23012357 DOI: 10.1074/jbc.M112.413427]

32   **Gallardo-Madueño R**, Leal JF, Dorado G, Holmgren A, López-Barea J, Pueyo C. In vivo transcription of nrdAB operon and of grxA and fpg genes is triggered in Escherichia coli lacking both thioredoxin and glutaredoxin 1 or thioredoxin and glutathione, respectively. *J Biol Chem* 1998; **273**: 18382-18388 [PMID: 9660805]

33   **Prieto-Alamo MJ**, Jurado J, Gallardo-Madueno R, Monje-Casas F, Holmgren A, Pueyo C. Transcriptional regulation of glutaredoxin and thioredoxin pathways and related enzymes in response to oxidative stress. *J Biol Chem* 2000; **275**: 13398-13405 [PMID: 10788450]

34   **Gon S**, Faulkner MJ, Beckwith J. In vivo requirement for glutaredoxins and thioredoxins in the reduction of the ribonucleotide reductases of Escherichia coli. *Antioxid Redox Signal* 2006; **8**: 735-742 [PMID: 16771665]

35   **Meister A**. Glutathione metabolism and its selective modification. *J Biol Chem* 1988; **263**: 17205-17208 [PMID: 3053703]

36   **Bushweller JH**, Billeter M, Holmgren A, Wüthrich K. The nuclear magnetic resonance solution structure of the mixed disulfide between Escherichia coli glutaredoxin(C14S) and glutathione. *J Mol Biol* 1994; **235**: 1585-1597 [PMID: 8107093]

37   **Vivancos PD**, Dong Y, Ziegler K, Markovic J, Pallardó FV, Pellny TK, Verrier PJ, Foyer CH. Recruitment of glutathione into the nucleus during cell proliferation adjusts whole-cell redox homeostasis in Arabidopsis thaliana and lowers the oxidative defence shield. *Plant J* 2010; **64**: 825-838 [PMID: 21105929 DOI: 10.1111/j.1365-313X.2010.04371.x]

38   **Dethlefsen LA**, Lehman CM, Biaglow JE, Peck VM. Toxic effects of acute glutathione depletion by buthionine sulfoximine and dimethylfumarate on murine mammary carcinoma cells. *Radiat Res* 1988; **114**: 215-224 [PMID: 3375425]

39   **Markovic J**, Mora NJ, Broseta AM, Gimeno A, de-la-Concepción N, Viña J, Pallardó FV. The depletion of nuclear glutathione impairs cell proliferation in 3t3 fibroblasts. *PLoS One* 2009; **4**: e6413 [PMID: 19641610 DOI: 10.1371/journal.pone.0006413]

40   **Rojas E**, Valverde M, Kala SV, Kala G, Lieberman MW. Accumulation of DNA damage in the organs of mice deficient in gamma-glutamyltranspeptidase. *Mutat Res* 2000; **447**: 305-316 [PMID: 10751614]

41   **Yoo MH**, Xu XM, Carlson BA, Patterson AD, Gladyshev VN, Hatfield DL. Targeting thioredoxin reductase 1 reduction in cancer cells inhibits self-sufficient growth and DNA replication. *PLoS One* 2007; **2**: e1112 [PMID: 17971875]

42   **Padovani D**, Mulliez E, Fontecave M. Activation of class III ribonucleotide reductase by thioredoxin. *J Biol Chem* 2001; **276**: 9587-9589 [PMID: 11266436]

43   **Logan DT**, Mulliez E, Larsson KM, Bodevin S, Atta M, Garnaud PE, Sjoberg BM, Fontecave M. A metal-binding site in the catalytic subunit of anaerobic ribonucleotide reductase. *Proc Natl Acad Sci USA* 2003; **100**: 3826-3831 [PMID:

12655046]

44 **Cerqueira NM**, Fernandes PA, Ramos MJ. Ribonucleotide reductase: a critical enzyme for cancer chemotherapy and antiviral agents. *Recent Pat Anticancer Drug Discov* 2007; **2**: 11-29 [PMID: 18221051]

45 **Shao J**, Zhou B, Chu B, Yen Y. Ribonucleotide reductase inhibitors and future drug design. *Curr Cancer Drug Targets* 2006; **6**: 409-431 [PMID: 16918309]

46 **Osterman Golkar S**, Czene S, Gokarakonda A, Haghdoost S. Intracellular deoxyribonucleotide pool imbalance and DNA damage in cells treated with hydroxyurea, an inhibitor of ribonucleotide reductase. *Mutagenesis* 2013; **28**: 653-660 [PMID: 24078015 DOI: 10.1093/mutage/get044]

47 **Aye Y**, Long MJ, Stubbe J. Mechanistic studies of semicarbazone triapine targeting human ribonucleotide reductase in vitro and in mammalian cells: tyrosyl radical quenching not involving reactive oxygen species. *J Biol Chem* 2012; **287**: 35768-35778 [PMID: 22915594 DOI: 10.1074/jbc.M112.396911]

48 **Piulats JM**, Jiménez L, García del Muro X, Villanueva A, Viñals F, Germà-Lluch JR. Molecular mechanisms behind the resistance of cisplatin in germ cell tumours. *Clin Transl Oncol* 2009; **11**: 780-786 [PMID: 20045784]

49 **Larsen IK**, Cornett C, Karlsson M, Sahlin M, Sjöberg BM. Caracemide, a site-specific irreversible inhibitor of protein R1 of Escherichia coli ribonucleotide reductase. *J Biol Chem* 1992; **267**: 12627-12631 [PMID: 1618768]

50 **Hurta RA**, Wright JA. Alterations in the activity and regulation of mammalian ribonucleotide reductase by chlorambucil, a DNA damaging agent. *J Biol Chem* 1992; **267**: 7066-7071 [PMID: 1551913]

51 **Lee Y**, Vassilakos A, Feng N, Jin H, Wang M, Xiong K, Wright J, Young A. GTI-2501, an antisense agent targeting R1, the large subunit of human ribonucleotide reductase, shows potent anti-tumor activity against a variety of tumors. *Int J Oncol* 2006; **28**: 469-478 [PMID: 16391803]

52 **Zhang M**, Wang J, Yao R, Wang L. Small interfering RNA (siRNA)-mediated silencing of the M2 subunit of ribonucleotide reductase: a novel therapeutic strategy in ovarian cancer. *Int J Gynecol Cancer* 2013; **23**: 659-666 [PMID: 23466567 DOI: 10.1097/IGC.0b013e318287e2b3]

53 **Ferdinandi ES**, Vassilakos A, Lee Y, Lightfoot J, Fitsialos D, Wright JA, Young AH. Preclinical toxity and toxicokinetics of GTI-2040, a phosphorothioate oligonucleotide targeting ribonucleotide reductase R2. *Cancer Chemother Pharmacol* 2011; **68**: 193-205 [PMID: 20886212 DOI: 10.1007/s00280-010-1473-z]

54 **Ohrström M**, Popović-Bijelić A, Luo J, Stenmark P, Högbom M, Gräslund A. Inhibition of chlamydial class Ic ribonucleotide reductase by C-terminal peptides from protein R2. *J Pept Sci* 2011; **17**: 756-762 [PMID: 21976435 DOI: 10.1002/psc.1399]

55 **Cooperman BS**, Gao Y, Tan C, Kashlan OB, Kaur J. Peptide inhibitors of mammalian ribonucleotide reductase. *Adv Enzyme Regul* 2005; **45**: 112-125 [PMID: 16054677]