A dityrosyl-diiron radical cofactor center is essential for human ribonucleotide reductases

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Abstract
Ribonucleotide reductase catalyzes the reduction of ribonucleotides to deoxyribonucleotides for DNA biosynthesis. A tyrosine residue in the small subunit of class I ribonucleotide reductase harbors a stable radical, which plays a central role in the catalysis process. We have discovered that an additional tyrosine residue, conserved in human small subunits hRRM2 and p53R2, is required for the radical formation and enzyme activity. Mutations of this newly identified tyrosine residue obliterated the stable radical and the enzymatic activity of human ribonucleotide reductases shown by electron paramagnetic resonance spectroscopy and enzyme activity assays. Three-dimensional structural analysis reveals for the first time that these two tyrosines are located at opposite sides of the diiron cluster. We conclude that both tyrosines are necessary in maintaining the diiron cluster of the enzymes, suggesting that the assembly of a dityrosyl-diiron radical cofactor center in human ribonucleotide reductases is essential for enzyme catalytic activity. These results should provide insights to design better ribonucleotide reductase inhibitors for cancer therapy.

Introduction
Ribonucleotide reductase catalyzes the reduction of ribonucleotides to the corresponding deoxyribonucleotides in all living cells. This reaction requires protein-derived radicals for DNA biosynthesis and repair (1, 2). Ribonucleotide reductases have been grouped into three classes based on their primary radical. Class I ribonucleotide reductase, found in eukaryotes, prokaryotes, and viruses, is a 1:1 protein complex that features two homodimers, R1 and R2. The R1 large subunit contains multiple binding sites for catalysis and regulation. The R2 small subunit provides a tyrosyl free radical and a neighboring diiron cluster essential for nucleotide reduction. Class II ribonucleotide reductase, existing mainly in bacteria, contains a transient 5'-deoxyadenosyl radical. Class III ribonucleotide reductase harbors a stable glycyl radical in anaerobic bacteria (1).

Human ribonucleotide reductase, a class I enzyme, has two small subunit homologous, hRRM2 and p53R2. p53R2 is a recently identified ribonucleotide reductase small subunit (1, 3) that is directly regulated by tumor suppressor p53 protein (1, 3). It is believed that p53 plays a crucial role in G1-G2 phase of the cell cycle, in apoptosis, and in nucleotide supply regulation during DNA repair (4). It has been proposed that p53R2 interacts with p53 to form a protein-protein complex (5). In response to genomic stress, p53 releases p53R2 and induces transcription of p53R2 for later use, whereas the free p53R2 binds to R1 for prompt DNA repair (4). Enzymatic properties in response to iron chelators, radical quenchers, and other ribonucleotide reductase inhibitors have been studied for p53R2 and hRRM2 (6). The essential role of ribonucleotide reductase in DNA biosynthesis and repair marks ribonucleotide reductase as a target for drugs designed to inhibit tumor cell growth in therapy.

For a long time, much effort has been given to design ribonucleotide reductase inhibitors for cancer therapy. However, an efficient inhibitor has yet to be found partially due to the lack of understanding of ribonucleotide reductase structure, especially concerning the tyrosyl diiron reaction center in small subunit R2. The information on the structure and function of the redox center of R2 is largely based on studies on Escherichia coli ribonucleotide reductase. Murine, human, and E. coli ribonucleotide reductase are all class I enzymes. Murine ribonucleotide reductase share 98% homologous with human ribonucleotide reductase and 27% homologous with E. coli ribonucleotide reductase. The three-dimensional structures of the E. coli and mouse R2 proteins reveal that the radical/iron site and the environment of the two proteins are quite similar (7). Using E. coli ribonucleotide reductase as a model system for class I enzymes, it has been found that a stable tyrosyl radical (at Tyr 22 in E. coli) is generated with the assistance of the dinuclear iron cluster in R2 (7). The radical is then transferred to the R1 catalytic site via a long-range proton-coupled electron transfer pathway and plays an essential role in deoxynucleotide formation (8).
Ribonucleotide reductase is an iron-dependent enzyme. Its small subunit contains a dinuclear iron cluster, which is a prerequisite for enzyme function. The iron site is involved in the generation and stabilization of the radical. Significant differences between *E. coli* and murine ribonucleotide reductases have been observed (9, 10). It has been proposed that a narrow hydrophobic channel allows oxygen and other small molecules to have direct access to the iron site in mice (11). This channel is blocked in *E. coli*. This more "open" structure makes mouse R2 more sensitive toward reductants, radical scavengers, and iron chelators (12). Furthermore, unlike that of *E. coli* R2, the radical iron center in mouse is labile. The mouse protein loses 50% of its iron after 30 minutes at physiologic temperature (9, 12). Electron paramagnetic resonance (EPR) study indicated a stronger magnetic interaction between the radical and the iron center in mouse R2 than in *E. coli* R2 (13). Effects of the tyrosyl radical on the structure and reactivity of the diferric center have been studied on both mouse and *E. coli* R2. The presence of the radical significantly affects both the structure and the reactivity of the iron site (14). In addition, a recently identified class Ic *Chlamydia* ribonucleotide reductase was found lacking the tyrosyl radical site, instead yielding an iron-coupled radical on reconstitution (15).

In our present work, we discovered that there are two (not one) tyrosine residues required for the formation of the stable radical and the diiron cluster of human ribonucleotide reductases. These observations are made using site-directed mutagenesis followed by enzyme activity assay and EPR spectroscopy. Structural models of hRRM2 and p53R2 reveal that these two tyrosine residues (Tyr<sup>162</sup> and Tyr<sup>138</sup> in hRRM2 and Tyr<sup>138</sup> and Tyr<sup>124</sup> in p53R2) are located at opposite sides of the diiron cluster, suggesting assembly of a dityrosyl-diiron radical cofactor center. The additional tyrosine residue could contribute to the observed discrepancies between mouse and *E. coli* ribonucleotide reductases regarding the radical iron center. This new finding of the dityrosyl-diiron radical cofactor center in human ribonucleotide reductases is essential for tyrosyl radical formation and the initiation of nucleotide reduction, specifically in class I ribonucleotide reductase, and may provide new model for antitumor and antiviral drug discovery.

**Materials and Methods**

**Plasmids**

The T7 RNA polymerase-responsive vector pET28a (EMD Biosciences Novagen, San Diego, CA) containing the cDNA encoding for hRRM2 and p53R2 protein was used for site-directed mutagenesis. Cloning procedures are conducted according to previous experiments (6).

**Oligonucleotide-Directed Mutagenesis**

PCR using pET-hRRM2 or pET-p53R2 as a template carried out mutation of Tyr<sup>162</sup> in hRRM2 to Phe<sup>162</sup> and Tyr<sup>124</sup> in p53R2 to Phe<sup>124</sup>. The primer for hRRM2 Y162F was 5'-ATTACAGAGCCCGCTTTTCTGGTTTC-CCAAATTGCATGGA-3', and the primer for p53R2 Y124W was 5'-GGTTCAAGGGCTGCTTTTTTTGGTTTCAAAATTCTCATCAGG-3' (boldface letters denote the mutated codon). PCR was conducted according to manufacturer’s protocol (Stratagene, La Jolla, CA). Each construct was transfected into *E. coli* strain BL21(DE3) (Stratagene), which enables isopropyl- β-D-galactopyranoside–induced overexpression of mutated p53R2 and hRRM2.

**Protein Expression and Purification**

Native and mutant hRRM2 and p53R2 were expressed in BL21(DE3) bacteria and then purified according to a previously published protocol (6). The same procedure was used for both proteins.

**In vitro Activity Assay**

The activities of hRRM2/hRRM1 and p53R2/hRRM1 were measured using a modified [3H]CDP reduction assay according to a previously published protocol (17).

**EPR Spectra**

X-band EPR spectra were measured with a Bruker EMX spectrometer equipped with an Oxford helium cryostat. Purified samples of hRRM2 and p53R2 proteins were frozen in liquid nitrogen before insertion in the cavity. Instrumental variables were as follows: *T* = 20 K; microwave frequency, 9.376 GHz; microwave power, 0.5 mW; modulation amplitude, 4 gauss; and modulation frequency, 100 kHz.

**Atomic Absorption Spectrometry**

Purified proteins were thoroughly washed by demineralized water and then diluted with 0.00005 N HNO<sub>3</sub>. Regenerated protein samples were prepared by 30 minutes of incubation with 15-fold FeCl<sub>3</sub> under room temperature followed by thorough washing. Measurement of iron content was done according to a previously published protocol (6).

**Models of hRRM2 and p53R2**

Three-dimensional models of hRRM2 (45 kDa) and p53R2 (43 kDa) were built using Composer in SYBYL 6.9.2 (Tripos, St. Louis, MO) based on a X-ray crystal structure of murine small subunit (PDB ID 1W69, 9) and evaluated by SYBYL Protable. Ramachandran plot, local geometry, and location of buried polar residue and exposed nonpolar residues were examined. Structures were refined by a series of energy minimization steps using AMBER all-atom force field.

**Results**

**Identification of Another Tyrosine Using EPR Spectroscopy**

Sequence alignment analysis of six class I small subunit proteins was done (Fig. 1). There are eight conserved tyrosine residues in hRRM2 and p53R2, and each was mutated to phenylalanine using site-directed mutagenesis. Wild-type and mutant proteins were purified from the cell lysate using a single chromatographic step. The amount of...
bound iron in the purified native and mutant proteins was characterized by atomic absorption spectrometry analysis. EPR spectroscopy was used to identify and characterize free radicals. EPR spectra (9 GHz) of native hRRM2 and p53R2 are virtually identical to those reported previously for mouse R2 (Fig. 2), indicating that the dihedral angle between the tyrosine C=H bond and the axis perpendicular to the aromatic ring are close (18). Quantitative EPR measurements gave 1.230 and 0.816 radicals per dimer of hRRM2 and p53R2, respectively (Table 1).

Mutants Y176F in hRRM2 and Y138F in p53R2 (will be called Y176F-M2 and Y138F-p53R2) gave EPR silent proteins (Fig. 2), suggesting that Tyr176-M2 and Tyr138-p53R2 are residues hosting the stable radical in hRRM2 and p53R2, respectively. This is in accord with previous studies in E. coli and mouse enzymes. Unexpectedly, Y162F-M2 and Y124F-p53R2 mutants are EPR silent as well. Mutations of either one of the two tyrosines (Tyr162-M2 and Tyr176-M2; Tyr124-p53R2 and Tyr138-p53R2) obliterated the stable tyrosyl radical, showing that both of the tyrosine residues are crucial for the formation of the stable tyrosyl radical. No significant changes were observed in EPR spectra of the other six tyrosine mutants, signifying that these tyrosine residues are not essential for radical formation (data not shown).

The Additional Tyrosine Is Required for Enzymatic Activity of Human Ribonucleotide Reductases

The enzymatic activities of the native and mutated hRRM2 and p53R2 proteins were measured in the presence of an excess of pure recombinant human R1 proteins by the [3H]CDP reduction assay. Both iron-regenerated and nonregenerated proteins were tested for enzymatic activity. No significant differences were observed between the two (data not shown). Consistent with the EPR results, no activities were detected in Y138F and Y124F mutants in p53R2 (Table 1), indicating both Tyr124 and Tyr138 are required for the enzymatic activity of p53R2. However, minimal activities in Y176F (<3.3% of that of the native protein) and Y162F (<3.7% of that of the native protein) of hRRM2 were obtained. The low activity found in the Y176F-M2 mutant suggests that there is another residue responsible for substrate reduction, similar to previous studies in E. coli and mouse (16, 19, 20). The complete lack of activity in double mutant Y176F/Y162F (Table 1) shows that both residues are necessary for human ribonucleotide reductase function. The inactive double mutant also indicates that only the two tyrosine residues of interest are responsible for the trace activity observed in the Y176F and Y162F mutants; neither other residues nor contamination by unseparated wild-type protein are responsible.

In an effort to further differentiate and compare the two tyrosyl radicals, we conducted an additional site-directed mutagenesis study. In this study, each of the two tyrosines was mutated to a tryptophan, which is another redox-active amino acid similar to tyrosine. Tryptophan substitution of either Y176W-M2 or Y162W-M2 gives EPR silent proteins (Fig. 2). This observation reinforces the idea that both of the two tyrosines are critical in radical formation. In contrast to phenylalanine mutations (Y176F and Y162F), tryptophan mutants (Y176W and Y162W) are completely enzymatically inactive (Table 1). This difference is due to disparities in the radical harboring ability of phenylalanine and tryptophan. The radical decays faster with a tryptophan substitution than with a nonradical phenylalanine substitution. This interpretation is supported by the observation in that the turnover of substrate per tryptophan mutant (Y177W in mouse) is less than that of phenylalanine.

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![Figure 1. Structure-based sequence alignment of the class I R2s from E. coli R2, Chlamydia R2, yeast R2, mouse R2, hRRM2, and p53R2 protein sequences.](image-url)
mutants (Y177F in mouse; ref. 21). Tryptophan substitution of Y138W-p53R2 gives EPR silent and enzymatically inactive protein as well. Interestingly, Y124W-p53R2 retained about one third of the radical content and enzyme activity of the native protein due to structural differences between hRRM2 and p53R2.

The Y369F-M2 or Y331F-p53R2 mutants gave the typical EPR signal (Fig. 2) yet have no detectable enzymatic activity (Table 1). This tyrosine aligns with Tyr370 in mouse/Tyr356 in E. coli, which is a part of the long-range electron transfer pathway at the R1-R2 interface (22, 23). Therefore, mutation of this residue inhibits radical transfer and hence enzymatic activity but not stable radical formation in small subunits. Our results confirm that this tyrosine is part of the electron transfer pathway of human ribonucleotide reductases as well.

Effect of the Newly Identified Tyrosine on Diiron Center Stability of Human R2 Proteins

To further study the effects of the newly identified tyrosine on the diiron center, iron was quantified using atomic absorption spectrometric measurements on purified proteins before iron regeneration (6). Interestingly, both Y138F-p53R2 and Y124F-p53R2 mutants lost half the iron content of the wild-type protein, indicating that the stability of the diiron site has been disturbed (Table 2). Each mutation (Y138F-p53R2 or Y124F-p53R2) seems to contribute to the loss of up to one iron per diiron center. Mutations on either tyrosine residues render mirroring effects on the iron content of ribonucleotide reductases as well.

Structural Models of hRRM2 and p53R2

Because no human R2 crystal structure is available to date, three-dimensional homology models of hRRM2 and p53R2 were built based on a recently published X-ray crystal structure of murine p53R2 (PDB ID 1W69, 9). Sequence alignment indicates that hRRM2 and p53R2 share high homology, 95.5% and 81.2%, respectively, with the murine R2 within 288 amino acids ranging from residues Asn 65 to Glu352 in 1W69. The models were evaluated using the SYBYL Protomodule to assure the accuracy of the protein structures.

As the X-ray structure of the murine R2 (1W69) would suggest, the dinuclear iron center is proposed to be located in a four-helix bundle in p53R2 and hRRM2. The hRRM2 structure model (we take hRRM2 as an example; p53R2 gives similar results) shows that each iron atom is coordinated by four ligands: His172, Asp138, Glu169, and Glu 266 for Fe1 and His269, Glu232, Glu266, and Glu 169 for Fe2 (Fig. 3). The two carboxylates, Glu 169 and Glu 266, bridge the two iron ions, which are separated by 3.4 Å. The two tyrosines are located at opposite sides of the dinuclear iron cluster (Fig. 3). The hydroxyl oxygen of Tyr176-M2 is 5.6 Å from Fe1 and 8.7 Å from Fe2; The hydroxyl oxygen of Tyr162-M2 is 10.8 Å from Fe2 and 13.9 Å from Fe2. The long distance between Tyr162-M2 and Fe2 suggests no direct interaction between the two parts. The model reveals that the two tyrosines are in slightly different environments. Tyr176 is surrounded mostly by hydrophobic leucines and isoleucines on one side but has access to hydrophilic iron ligands on the other. Similarly, Tyr162 is enclosed by hydrophobic phenylalanines and is closest to a hydrophilic residue near the iron cluster. The hRRM2 structural model indicates that, although both
tyrosine residues are buried in the enzyme interior, Tyr162-M2 (~8.8 Å from protein surface) sits closer to the surface than Tyr176-M2 (~10.3 Å from the surface), making the former more accessible.

**Radical Simulation on the Newly Identified Tyrosine**

Tyr162-M2/Tyr124-p53R2 is similar to Tyr176-M2/Tyr138-p53R2 with regard to its importance for human ribonucleotide reductase function. However, how much Tyr162-M2/Tyr124-p53R2 and Tyr176-M2/Tyr138-p53R2 alike and whether Tyr162-M2/Tyr124-p53R2 possesses a radical, transient or stable, is a very interesting and an open question. We used an EPR spectrum simulation method to find that Tyr162-M2 possibly harbors a radical with an EPR spectrum very similar to that of Tyr177. This method, established by Svistunenko and Cooper, predicts the EPR spectra of tyrosyl radical sites in a protein by comparing the rotational/dihedral angle (θ) of a tyrosine phenoxyl ring with the simulated value from the shape of the EPR spectra of tyrosyl radicals (24). Svistunenko and Cooper simulated the rotational angle of Tyr177 from the structures reveal that human Tyr162 and murine Tyr163 are each surrounded by 10 identical amino acid residues, which assure that the calculations based on the murine structure can be applied to the human protein. Structure calculation analysis indicates that both Tyr162-M2 and Tyr176-M2 could have similar dihedral angles, which suggests that the EPR signals of these two tyrosines may overlap or that Tyr162-M2/Tyr124-p53R2 gives a transient radical that decays before detection.

**Discussion**

Mammalian Ribonucleotide Reductases Possess Different Radical Generation Mechanism from *E. coli* Ribonucleotide Reductase

*E. coli* ribonucleotide reductase has been the model organism for class I ribonucleotide reductase enzymes. It is believed that the radical generation mechanism of all class I enzymes follow similar routes. However, sequence

<table>
<thead>
<tr>
<th>Table 1. Ribonucleotide reductase activity and radical content of the wild-type and mutant in small subunits</th>
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<tbody>
<tr>
<td>p53R2</td>
</tr>
<tr>
<td>Wild-type</td>
</tr>
<tr>
<td>Y124F</td>
</tr>
<tr>
<td>Y138F</td>
</tr>
<tr>
<td>Y124F/Y138F</td>
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<tr>
<td>Y331F</td>
</tr>
<tr>
<td>Y124W</td>
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<tr>
<td>Y138W</td>
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NOTE: Iron quantification is done on freshly purified protein using atomic absorption spectrometry. The activity of the protein was presented as percentage of formation of [3H]CDP (see Materials and Methods). The enzyme activity was measured in the presence of 0.5 μmol/L R1 protein and 2 μmol/L hRRM2 or p53R2 protein with a total sample volume of 100 μL. Average of two to three determinations with deviations less than 0.3. ND, not detectable.

The radical content was quantified against a standard solution of 1 mmol/L CuSO₄ in 50 mmol/L EDTA by double integration of spectra registered at nonsaturating microwave levels by standard Bruker software.

<table>
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<th>Table 2. Iron quantification of freshly purified proteins</th>
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<tr>
<td>p53R2</td>
</tr>
<tr>
<td>Wild-type</td>
</tr>
<tr>
<td>Y124F</td>
</tr>
<tr>
<td>Y138F</td>
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<tr>
<td>Y124F/Y138F</td>
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<tr>
<td>Y331F</td>
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NOTE: Iron quantification is done on freshly purified protein using atomic absorption spectrometry.

* Proteins (μmol/L) are called dimers. 

Method (Table 3). Only Tyr177 and Tyr163 have rotational angles that agree well with the simulated result (θ = 3°). Our calculation for the rotational angle of Tyr177 (θ = 18.3°) matches Svistunenko and Cooper’s result. Yet, remarkably, the rotation angle of Tyr163 (θ = 6.5°), in alignment with human Tyr162, was found closest to the simulated stable tyrosyl radical value (θ = 3°). According to this method, a radical at Tyr163 should give an EPR spectrum similar to that of Tyr177. Comparisons between the structures reveal that human Tyr162 and murine Tyr163 are each surrounded by 10 identical amino acid residues, which assures that the calculations based on the murine structure can be applied to the human protein. Structure calculation analysis indicates that both Tyr162-M2 and Tyr176-M2 could have similar dihedral angles, which suggests that the EPR signals of these two tyrosines may overlap or that Tyr162-M2/Tyr124-p53R2 gives a transient radical that decays before detection.
alignment of various ribonucleotide reductases reveals that, although the sequences of mammalian small subunits are very similar (80% homology), they are distant from other members of class I enzymes (e.g., 25% homology with \textit{E. coli}). The amino acids close to Fe2 of the diferric cluster differ significantly between murine and \textit{E. coli} R2 (11). In particular, \textit{E. coli} R2 lacks a tyrosine at position 108 near the Fe2 site, which aligns with Tyr163 in mouse R2 and Tyr124-p53R2 in human R2 (Fig. 1). It is interesting to note that the transient formation of some tryptophan radicals in close proximity to Fe2 has been observed in Y122F \textit{E. coli} (16, 21, 25), giving 1% to 2% of wild-type activity (26). Intriguingly, a new R2 subclass (class Ic) defined in \textit{Chlamydia} ribonucleotide reductase (15) lacks the canonical tyrosyl radical site (near Fe1), but an EPR signal with a shorter life span was detected. Structural alignment indicates that two tyrosine residues in the vicinity of the Fe2 site (Tyr124 and Tyr162) in \textit{Chlamydia} R2 could be candidates for an alternative tyrosyl radical.

The high accessibility of the radical diiron cluster center could make mammalian ribonucleotide reductases more efficient in radical generation, reactivity, and transportation (9). Radical intermediate of the murine R2 could be induced by mild chemical reduction in 30% yield, whereas \textit{E. coli} only yielded \sim 5% under drastic conditions (12). Furthermore, the additional tyrosine could supply the fourth electron in the radical reconstitution reaction, where four electrons are required for dioxygen reduction and only three are provided by the protein, in which two are from ferrous ions and one from the known tyrosyl radical (20).

**Comparison of p53R2 and hRRM2**

Studies of enzymatic properties in response to iron chelators, radical quenchers, and other ribonucleotide reductase inhibitors revealed differences in characteristics between p53R2 and hRRM2 (6). In this study, both similarities and differences were observed between the two human ribonucleotide reductase small subunits, p53R2 and hRRM2. p53R2 is homologous to hRRM2 with 83% sequence identity. The dissimilarities between p53R2 and hRRM2 are due to their structural differences. Our conclusions, despite the differences between the two small subunits, regarding the formation of a tyrosyl radical center in hRRM2 are applicable to p53R2 as well.

Here, we found for the first time that there is another tyrosine residue (Tyr162-M2/Tyr124-p53R2) involved in the stable radical generation process. A diferryl-diiron radical cofactor center, which is essential for enzymatic activity, is proposed in human ribonucleotide reductases. Human ribonucleotide reductase is an important therapeutic target for cancer treatment. Current cancer therapy uses several ribonucleotide reductase inhibitors to limit the rate of DNA replication and oncogenic cell proliferation. The recent resurgence of interest in ribonucleotide reductase, as an anticancer target, propels the need for better protein-specific inhibitors that would reduce toxicity and increase efficacy of cancer therapy. To do so, a thorough understanding of human ribonucleotide reductase is crucial. This discovery of the formation of a diferryl-dityrosyl radical cofactor center in human ribonucleotide reductases can contribute to the further

**Table 3. Rotational angles of the tyrosine residues in mouse R2 and hRRM2 determined using Svistunenko and Cooper method**

<table>
<thead>
<tr>
<th>Tyrosine (mouse)</th>
<th>Tyrosine (hRRM2)</th>
<th>Angle (θ)</th>
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<tbody>
<tr>
<td>88</td>
<td>(87)</td>
<td>27.8</td>
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<td>95</td>
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<td>98.6</td>
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<td>163</td>
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<td>177</td>
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<td>184</td>
<td>(183)</td>
<td>164.2</td>
</tr>
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<td>193</td>
<td>(192)</td>
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<tr>
<td>222</td>
<td>(221)</td>
<td>84.2</td>
</tr>
<tr>
<td>324</td>
<td>(323)</td>
<td>77.4</td>
</tr>
</tbody>
</table>

**NOTE:** Phenoxyl ring rotation angles (θ) of tyrosine residues in mouse R2 were calculated using the equation described by Svistunenko and Cooper and based on the mouse crystal structure 1W69 (PDB ID). Residue number of mouse R2 tyrosines and the corresponding hRRM2 are listed. The possible tyrosyl radical harboring residues are boldfaced.

Figure 3. Schematic drawing of the dityrosyl radical dinuclear iron cluster of human p53R2 and hRRM2.

Coordination of each iron ion is shown with ligands Asp100/Asp138, His134/His172 and Glu194/Glu232, His231, His269 and Glu131/Glu169 for Fe1 and Glu194/Glu169, His231, His269 and Glu131/Glu169 for Fe2. Glu131/Glu169 and Glu228/Glu266 bridge two iron. Residues were numbered as p53R2/hRRM2. The radical harboring sites Tyr124/Tyr162 and Tyr138/Tyr176 are shown with Tyr138/Tyr176 close to the Fe1 site and Tyr124/Tyr162 close to the Fe2 site.
elucidation of the fundamental mechanism of ribonucleotide reductases and will offer significant insight for the design of new ribonucleotide reductase inhibitors for cancer therapy.

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