Communication

Identification of a Novel Human Peroxiredoxin-6 (PRDX6) Promoter Region Sequence

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Abstract: Peroxiredoxin-6 (PRDX6) is an oxidative stress response protein with multiple roles under reactive oxygen species (ROS)-mediated conditions. The multifunctional cytoprotective role of the protein PRDX6 is to maintain cellular homeostasis and membrane integrity through intracellular ROS and phospholipid turnover. The PRDX6 promoter region has been reported in several biological regulations because of its key binding sites. However, the human PRDX6 promoter sequence is not yet fully understood. In this study, we discovered and characterized the proximal promoter sequence of human PRDX6. Interestingly, the identified human PRDX6 promoter was about 1 kb shorter than the one reported in the database. Further studies with the new human PRDX6 promoter analysis will help to understand intracellular ROS and phospholipid turnover to maintain cellular homeostasis. This report provides the human PRDX6 proximal promoter sequence.

Keywords: peroxiredoxin-6; promoter sequence; deletion; database; BAC clone

1. Introduction

Inflammation and age-related diseases are major pathophysiological conditions associated with elevated levels of reactive oxygen species. In these conditions, peroxiredoxins (PRDX1-6) play multiple roles, including cytoprotecting against oxidative stress, regulation of redox signaling, and innate immunity [1–4]. Among mammalian peroxiredoxins, PRDX6 can reduce phospholipid levels and short chain hydroxy peroxide levels through glutathione peroxidase (Gpx) activity.

In addition to the antioxidant activity of GSH peroxidase, PRDX6 also has acidic $Ca2^{+}$-independent phospholipase A2 activity [5–8]. Therefore, PRDX6 is considered a multifunctional oxidative stress response protein that multifunctionally maintains cellular homeostasis and membrane integrity through intracellular ROS and phospholipid turnover [7,9,10]. Moreover, a wide range of physiological and pathological PRDX6 associations signifies its involvement in various signaling pathways including interferon-γ (IFNγ) regulated by interleukin-18 binding protein (IL-18BP) [11]. Accumulating the evidence of in vitro and in vivo results showed that PRDX6 expression was correlated to ROS-mediated gene expressions such as transforming growth factor (TGF)β and NF-κB [12–18].

The PRDX6 promoter region contains multiple regulatory elements associated with antioxidant gene expression. These include the antioxidant response element (ARE), transcription factor Sp1, repressive Smad3 binding element (RSBE), tyrosine kinase with immunoglobulin-like and EGF-like domains 1 (TIE1), and NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) binding sites. Nonetheless, given the role of ROS as mediators of normal redox signaling, it is believed that fine-tuning of PRDX6 requires optimal coordination to avoid overshooting the desired beneficial effects. Disruption of the delicate redox balance is essential for the maintenance of normal cellular function. Therefore, PRDX6 and its promoter should be investigated to determine how PRDX6 activity changes under physiological and pathological conditions. However, the human PRDX6 promoter sequence and its transcriptional regulation have not been properly validated. Here, we discovered the human PRDX6 promoter sequence for the first time. Furthermore, we submit the identified sequences to the

database for correction of the annotated PRDX6 promoter region, providing a basis for further experiments required for valid human PRDX6 promoter sequences.

2. Materials and Method

2.1. Cell Culture

Human HepG2, PC3, HT29, and colo205 cell were obtained from American type culture collection (ATCC) and maintained according to ATCC instructions. These cells were used for genomic DNA extraction, followed by PCR analysis.

2.2. PCR and Cloning Constructs

Bacterial Artificial Chromosome (BAC) clone: RP3-436N22 was obtained from Children's Hospital Oakland Research Institute. PCR was performed to amplify the promoter region of PRDX6 with the flowing primer sets forward 5′ ATCGAGGCATAGAACCTTTCC (p1), 5′ TCCCACCTGACGAGAAACAC (p2), and 5′ CCCTTCACAAGAAGGAAA (p3) and reverse 5′ CGGTGGCCACTTACGAGTC (p4). Next, the longest PCR product was cloned using T&A cloning vector system (RBC, Taiwan) following the manufacturer's instructions [19]. The screened clone was confirmed by sequencing analysis (Cosmo Genetech, Seoul, Republic of Korea).

3. Results

3.1. PCR of Human Genomic DNA and IDENTIFICATION of the PRDX6 Promoter Sequence

First, we aimed to amplify the promoter region of PRDX6 from the genomic DNA of four human cell lines by PCR. However, a pair of forward primer (p)1 and reverse p5 failed to amplify PRDX6 promoter form human cell lines (Figure 1). Next, we obtain human BAC clone (RP3-436N22) to amplify the promoter region of PRDX6 using the BAC clone by PCR. The predicted size is 2410 base pair (bp), according to databank (accession number AL139142.10). In contrast, the resulting PCR product showed a shorter length of PRDX6 promoter region using forward p1 and reverse p4. The PCR product was approximately 1 kb in size (Figure 1B).

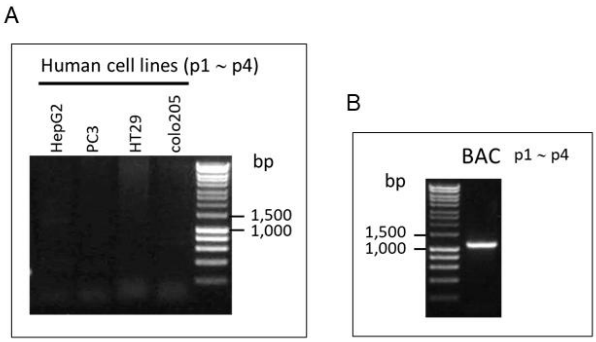

Figure 1. PCR of PRDX6 promoter using genomic DNA from human cell lines and BAC. (**A**) PCR was performed to clone the PRDX6 promoter using sequences known in the database using human genomic DNA extracted from four cell lines, HepG2, PC3, HT29, and colo205. No PCR products were observed. (**B**) PCR was performed to clone the PRDX6 promoter using BAC DNA, using the same forward and reverse primers as those used for the genomic DNA of the human cell line. PCR products were observed at 1 kb or longer.

The PCR product of p1 and p4 was cloned into a T&A cloning vector and was further analyzed with DNA sequencing. Surprisingly, the sequencing result revealed exactly 1153 bp and was submitted to the GenBank database (submission ID: 1476633) in Figure 2A. The identified new PRDX6 promoter 1257 bp compared to the known databank (accession number AL139142.10) and gray colored region 1153 bp is absent in new PRDX6 promoter from BAC clone (Figure 2B).

Figure 2. Cloning and sequencing of human PRDX6 promoter. (**A**) This is the DNA sequence of the PRDX6 promoter newly synthesized from BAC. It was 2410 bp in the database, but it turned out to be 1257 bp. There is a 1153 bp deletion between the yellow and green highlighted regions. A single point mutation A changed to G in the middle of the promoter highlighted in blue is shown in red bold. (**B**) The known PRDX6 promoter sequence present in the database is shown, with the deleted 1153 bp highlighted in gray. The change to G is caused by the single point mutation A highlighted in blue and indicated in bold red.

3.2. Alignment with Databank PRDX6 Promoter and PCR of BAC Genomic DNA with Different Forward Primers

The newly identified PRDX6 promoter 1257 bp was blasted in human gene bank and the result was shown in Figure 3. The alignment matched with AL139142 clone of chromosome 1q24.1–25.3. There was a single point mutation and A changed to G which was highlighted with red letter with yellow color. To confirm the identified new PRDX6 promoter, several forward primers were designed in Figure 4A and the exact location was depicted in Figure 4A. The forward primer, p1 and p2 present in newly identified PRDX6 region, but p3 is located only on the old PRDX6 promoter from databank (Figure 4A upper part). As we expected the forward primer p1 and p2 with reverse p4 amplified the size of 1257 bp and 995 bp PCR band, whereas forward p3 failed amplified PCR product that primer site is not present on new PRDX6 promoter (Figure 4B).

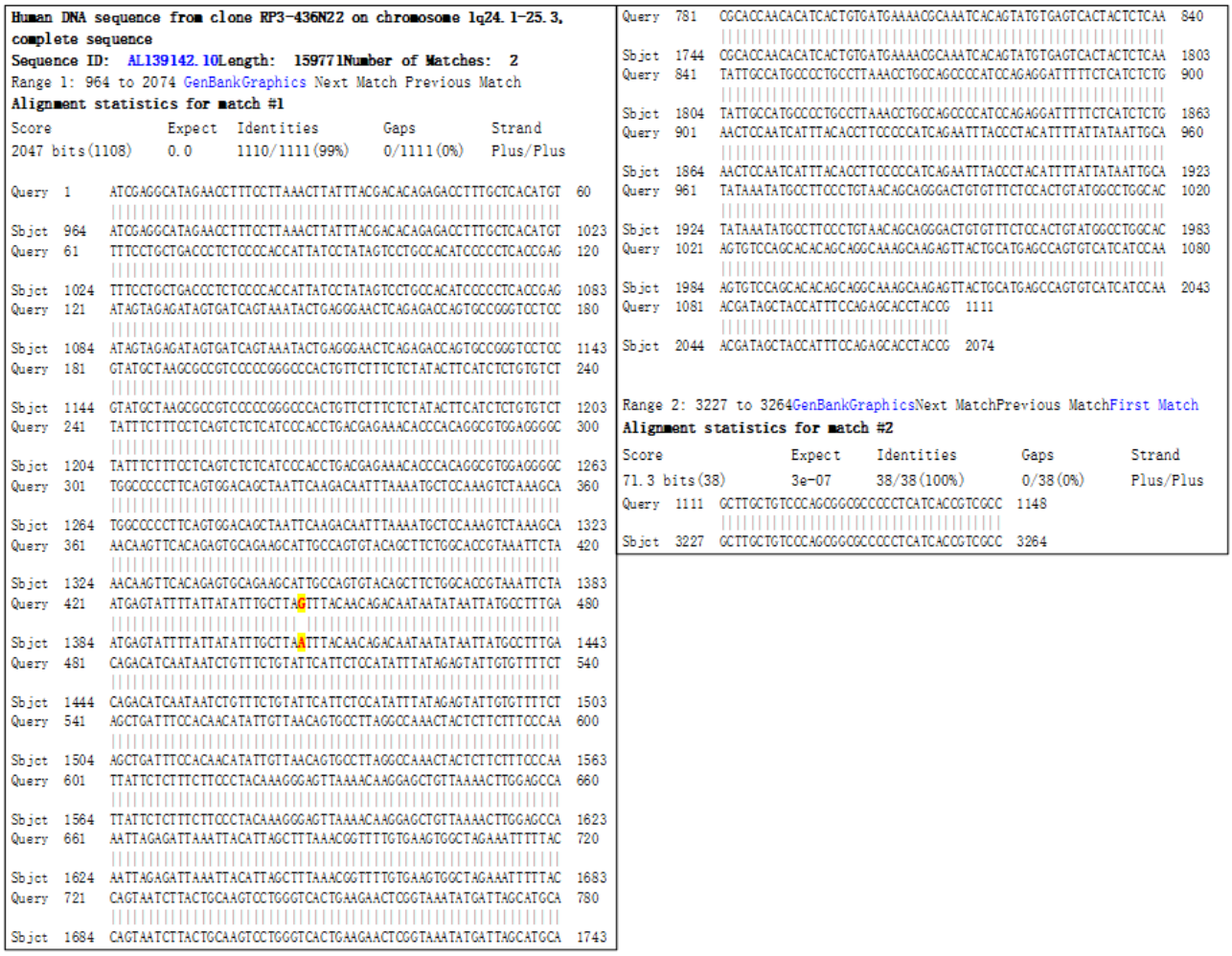

Figure 3. Alignment of the newly cloned human PRDX6 promoter**.** A novel human PRDX6 promoter sequence of 1273 bp was used for blasting the human genome database. As shown in the figure, this sequence is aligned with the accession number AL139142 of RP3-436N22 located on chromosome 1q24.1–25.3 (left and upper part). A deletion is found in a very short alignment stretch between 2074 bp and 3227 bp in the lower right. The single-point mutation, A to G, is highlighted in yellow and bolded in red.

The single point mutation A changed to G is indicated as −704 bp upstream from ATG of human *PRDX6* gene. The deletion occurred between 1111 bp and 2264 bp on databank AL139142 clone that shown in Figure 4A (lower part). The deleted region 1153 bp depicted with red dot line in BAC clone (Figure 4A upper part).

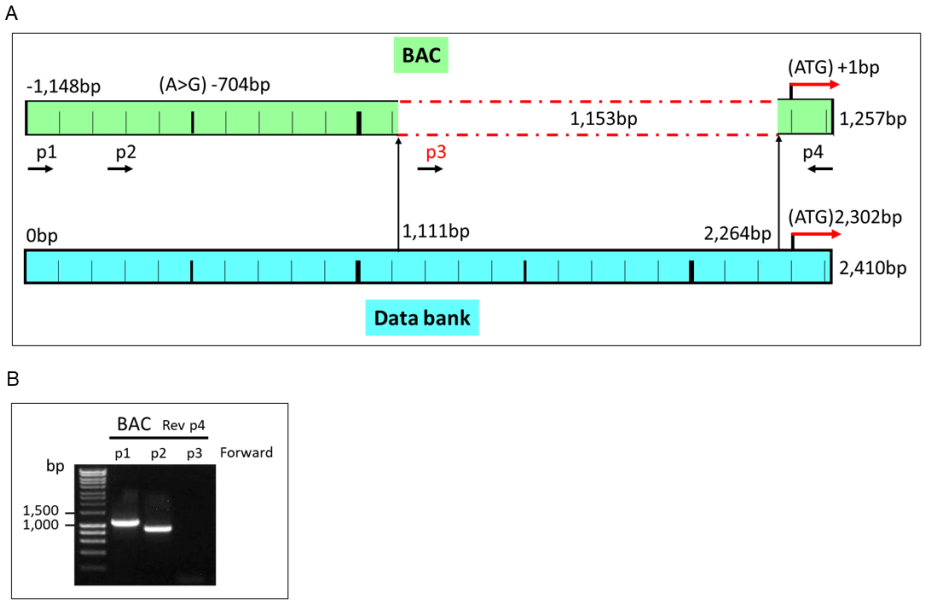

Figure 4. PRDX6 promoter diagram shows deletion of PRDX6 promoter and PCR using BAC**.** (**A**) The 2410 bp PRDX6 promoter from the database is shown at the bottom, and the 1257 bp PRDX6 promoter newly cloned from the BAC is shown at the top. The upper part, indicated by the dotted line, has deleted 1153 bp, while the lower part is between 1111 bp and 2264 bp. A > G mutation was located −704 bp of ATG start codon of PRDX6. (**B**) To confirm the deletion in BAC, we designed two forward primers: p2, which is present in both the novel promoter and the database PRDX6 promoter, and p3, which is absent from the novel promoter. As expected, when PCR was performed using the BAC, p1 and p2 present in the new promoter generated PCR products of the expected size, but p3 did not amplify any PCR product.

4. Discussion

PRDX6 is a pleiotropic protein that plays a key role in response to oxidative stress. It contains both peroxidase and phospholipase activities, and as a result, participates in health and disease conditions. Therefore, the fine regulation of PRDX6 expression is required to optimize ROS levels. However, its expression and molecular regulations are not completely understood. In this study, we identified the sequence of the human PRDX6 promoter and evaluated its activity. The newly identified promoter sequence was submitted to GenBank (submission ID: 1476633). Furthermore, the previous annotations, including length and coordination within the human genome, should be corrected on the database.

First, we attempted to isolate the human PRDX6 promoter from human genomic DNA isolated from human cell lines. However, we failed to clone it using the known human PRDX6 promoter sequence in the database. Therefore, we obtained the BAC clone of the human PRDX6 promoter and isolated the new human PRDX6 promoter. Interestingly, the length of the amplified DNA was 1153 bp shorter compared to the reference human PRDX6 promoter sequence in the database.

A recent study suggested the peroxidase activity of PRDX6 as an antioxidant after LPS induction through reducing short chain hydroperoxide. However, they observed an overall reduction in PRDX6 expression in cell lines and patient tissues [20]. This effect may explain the redox balance regulatory role of PRDX6 in accordance with the cell requirements and microenvironments to sustain homeostasis. Nevertheless, more studies are required to explore this event. It is also important to understand and explore the regulatory domains of the newly promoter region. Further studies are needed which will allow researchers to examine the subfunctions and how these sites may involve several pathological pathways. In this study, the identified PRDX6 proximal promoter sequence replaces the annotated wrong sequence in database.

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Abbreviations

ARE, Antioxidant response element; ATCC, American Type Culture Collection; BAC, Bacterial Artificial Chromosome; F, Forward; Gpx, glutathione peroxidase; IFNγ, interferon-γ IL-18BP, interleukin-18 binding protein; NF-κB, Nuclear factor kappa-light-chain-enhancer of activated B cells; PRDX6, peroxiredoxin-6; R, Reverse; ROS, Reactive oxygen species; RSBE, Repressive smad3 binding element; TGF, Transforming growth factor; TIE1, Tyrosine kinase with immunoglobulin-like and EGF-like domains 1.

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