Poly(ADP-ribose) polymerase-1 regulates vimentin expression in lung cancer cells

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Chu S, Xu H, Ferro TJ, Rivera PX. Poly(ADP-ribose) polymerase-1 regulates vimentin expression in lung cancer cells. Am J Physiol Lung Cell Mol Physiol 293: L1127–L1134, 2007. First published August 24, 2007; doi:10.1152/ajplung.00197.2007.—Vimentin is one of the mammalian intermediate filament proteins. It is expressed in cells of mesenchymal origin and is characteristic of proliferating cells at the fetal stage. During malignancy, vimentin expression is activated in certain lung epithelial cells. Examination of a group of lung cancer cells showed a marked difference in their vimentin expression. The difference in vimentin expression among lung cancer cells is due to differential regulation at the transcriptional level. Analysis of the vimentin promoter revealed a 102-bp promoter sequence that is important for promoter activity in a lung cancer cell line in which vimentin is strongly expressed. This promoter region interacts with poly(ADP-ribose) polymerase-1 (PARP-1), which is also a transcription regulator. Exogenous expression of PARP-1 increased vimentin promoter activity. A shortened PARP-1 without the COOH-terminal catalytic domain showed a similar promoter activation effect. Treatment of cells with H2O2 reduced PARP-1 and vimentin expression at the protein level. H2O2 also dose dependently suppressed vimentin promoter activity in cells overexpressing PARP-1. These results demonstrate that vimentin expression in lung cancer cells is regulated at the transcriptional level and that PARP-1 binds and activates the vimentin promoter independent of its catalytic domain and may play a role in H2O2-induced inhibition of vimentin expression.

INTERMEDIATE FILAMENTS are one of the three major cytoskeleton filaments in eukaryotic cells that help maintain cell shape and assist movement. Component proteins of intermediate filaments include cytokeratins, vimentin, and other filament proteins and accessory proteins. Vimentin is usually expressed in cells of mesenchymal origin. In addition to its roles in cell shape and movement, vimentin is also recognized as an indicator of cancer progression, because its appearance in certain vimentin-negative cells is often associated with cancer metastasis (39).

In a number of malignancies of epithelial origin, a transition accompanying the progression from local growth to metastasis occurs in which the epithelial cancer cells lose epithelial characteristics and acquire a mesenchymal phenotype [epithelial-mesenchymal transition (EMT)] (36). Cellular and molecular changes at EMT include the loss of cell-cell contacts, rearrangement of the cytoskeletal network, increased cell migration, expression of mesenchymal protein markers, and disappearance of epithelial protein markers. EMT is present in lung cancers (2). EMT also determines the sensitivity of lung cancer to certain anticancer treatment (47). More recently, EMT has been shown to occur in alveolar epithelial cells and to mediate idiopathic pulmonary fibrosis (42, 46). Vimentin is normally expressed in mesenchymal cells and is one of the marker proteins of EMT.

In normal lung epithelia, vimentin is expressed only in fetal bronchial epithelium, and this expression decreases with age (4). However, vimentin expression is increased in lung cancers, as well as many other cancers of epithelial origin (20, 38). The significance of the activation of vimentin expression is twofold. 1) Vimentin may exert direct or indirect effects on cell-cell contacts and cell migration. Although exact mechanisms remain unclear, evidence has been presented demonstrating an association between vimentin expression and increased invasiveness of cancer cells (22, 37). 2) Molecular events leading to the activation of vimentin expression may provide a window for accessing the regulatory mechanisms that control EMT and, thus, cancer progression.

As one of the mesenchymal markers, vimentin is associated with metastasis and prognosis of a number of cancers, such as breast cancer and papillary thyroid carcinoma (22, 37, 39, 41). In human lung, vimentin is known to be strongly expressed in some cancer cell lines. However, its expression in lung cancer has not been studied in detail since early reports in the 1980s (20, 38). Therefore, it remains unclear when or during which stage of lung cancer vimentin becomes positive. Blanco and colleagues (2) recently used a rat model to demonstrate sequential events leading to EMT in lung cancer. In this study, animals were injected with crystalline silica through the trachea and lung samples were analyzed at different time points. Vimentin-positive tumor cells were found in 35% of adenocarcinomas and 88% of squamous cell carcinomas, but not in hyperplastic and adenomatoid precursor lesions. In contrast, expression of E-cadherin, an epithelial marker, was significantly reduced in precursor lesions, as well as in late-stage tumors. These findings not only confirm the activation of vimentin expression in lung malignancies, they also indicate a subtle difference between EMT markers of E-cadherin and vimentin. The former disappears early in tumorigenesis, and the latter appears at late stages of tumor progression, which may signal a transition of cancer cells from local growth to metastasis. Although vimentin is well documented as a marker protein for EMT and cancer metastasis, its regulation in lung cancer cells is not well understood, and the mechanism that triggers vimentin expression in lung epithelial cells is unclear.

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Poly(ADP-ribose) polymerase-1 (PARP-1) is a member of the PARP enzyme family and a nuclear enzyme that, on binding to DNA, breaks, cleaves NAD$^+$ into nicotinamide and ADP-ribose, and polymerizes the latter onto target molecules, including histones, transcription factors, and itself. Functions of PARP-1 are multifold, are not well understood, and may vary depending on cell type. However, PARP-1 is known to mediate cell death by depleting ATP and NAD$^+$ (7), to facilitate cell survival via DNA repair (15), and to regulate gene transcription through interactions with transcription factors and promoters (10). Even with these few known functions, the overall role of PARP-1 in cells is complex, because it promotes cell survival and death. PARP-1 overexpression has been reported in cancer cells (26, 29) and may promote cancer cell survival and growth through enhanced DNA repair. PARP-1 overexpression may also increase cancer cell resistance to chemotherapeutic and radiotherapy (17, 49). Indeed, inhibition of PARP-1 has been found to enhance efficacy of anticancer therapies (6, 16).

Lung cancer cells show vastly different characteristics. In some lung cancer cells, vimentin is expressed at high levels; in others, vimentin expression is undetectable. PARP-1 maintains genomic integrity in cancer cells, which facilitates cancer growth. It is not known whether PARP-1 promotes cancer growth also through other means, namely, transcription regulation of cancer-related genes such as vimentin. Vimentin and PARP-1 have been implicated in the outcomes of lung cancer therapy (1, 19, 47). The interrelationship between these factors promotes cell survival via DNA repair (15), and to regulate gene transcription through interactions with transcription factors and promoters (10). Even with these few known functions, the overall role of PARP-1 in cells is complex, because it promotes cell survival and death. PARP-1 overexpression has been reported in cancer cells (26, 29) and may promote cancer cell survival and growth through enhanced DNA repair. PARP-1 overexpression may also increase cancer cell resistance to chemotherapeutic and radiotherapy (17, 49). Indeed, inhibition of PARP-1 has been found to enhance efficacy of anticancer therapies (6, 16).

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**MATERIALS AND METHODS**

*Cells and reagents.* Human lung epithelial cell lines HAE, H441, A549, WI-26, and Calu-3 (catalog nos. CRL-2170, HTB-174, CCL-185, CCL-95.1, and HTB-55, respectively) were cultured according to the protocols recommended by the American Type Culture Collection. Purified recombinant human PARP-1 was purchased from R&D Systems (Minneapolis, MN), a full-length human PARP-1 overexpression vector from OriGene (Rockville, MD), and acridine orange and propidium iodide from Sigma.

*Construction of plasmids.* Vimentin promoter-luciferase vectors were constructed by restriction deletions and cloning of PCR fragments. The 1.5-kb vimentin promoter clone was kindly provided by Dr. Gilles (University of Liege) (21). All clones were confirmed by DNA sequencing or restriction digestions.

A mutant PARP-1 expression vector, which codes for a shorter version of PARP-1 without the COOH-terminal catalytic domain, was constructed using *BamH* I digestion and self-ligation. This results in a deletion of 479 amino acid residues at the COOH terminus of the 1,014-residue full-length PARP-1 protein, which contains the catalytic domain, and the addition of a short 9-amino acid sequence (VPGSSDSPA). This vector was verified by DNA sequencing, and the shorter version of the PARP-1 protein (PARP-cat) was verified using immunoblotting (see Fig. 5A).

*Promoter-reporter gene assay and EMSA.* Promoter-reporter gene assay and EMSA were performed as previously described (11). Transfection efficiency was calibrated by cotransfection with a vector coding for a herpes simplex virus (HSV) thymidine kinase (TK) promoter-driven Renilla luciferase (Promega).

*Nuclear protein pull-down.* Cell nuclear extracts were prepared as described previously (11). Streptavidin-conjugated magnetic beads were purchased from Invitrogen. Biotinylated oligonucleotides were synthesized and annealed. The annealed double-stranded oligonucleotide was coupled to streptavidin-conjugated beads, which were used for incubation with the nuclear extract to pull down DNA-binding proteins. Nucleotide coupling and nuclear protein pull-down were performed according to the protocols recommended by the bead manufacturer.

**RESULTS**

Vimentin expression is markedly different in lung cancer cell lines. Cultured lung cancer cells show markedly different phenotypes, such as growth rate, cell-cell association, and protein markers, which may reflect metastatic potential or grade of malignancy. One of the indicators for malignancy and late-stage cancers is the presence of vimentin (22, 23, 33). The levels of vimentin expression in lung cancer cells are widely different, from below the detection limit to strongly positive (Fig. 1A).

In addition to expression at the protein level, we also examined vimentin mRNA levels using RT-PCR in HAE and H441 cells. Consistent with the immunoblotting data, vimentin mRNA is strongly expressed in HAE cells but nearly undetected in H441 cells (Fig. 1B). The strong expression of vimentin in the mRNA level in HAE cells indicates a role of the vimentin promoter. Vimentin promoter activity was then tested using the firefly luciferase reporter gene. Cells were transfected with a promoter-luciferase vector that contains a 1,463-bp vimentin promoter fragment. Transfection efficiency was calibrated with the expression of a cotransfected TK promoter-driven Renilla luciferase reporter. Our results show that the vimentin promoter is more than six times as active in HAE cells as in H441 cells (Fig. 1C). These results suggest that vimentin expression in lung epithelial cells is regulated at the transcriptional level and that its promoter plays an important role in this regulation.

A 102-bp promoter sequence is important for vimentin promoter activity. Because HAE expresses high levels of vimentin, we used HAE cells to investigate how vimentin promoter is regulated. A series of promoter-luciferase vectors

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with different lengths of vimentin promoter inserts were constructed (Fig. 2). Luciferase reporter experiments demonstrate that the full-length 1,463-bp fragment is one of the most active fragments (Fig. 2). Deletion analysis showed that sequences at the 3′/H11032 end and at the very 5′/H11032 end of the 1,463-bp promoter do not seem to have a major role in promoter activity. However, deletions of sequences around the PvuII site decreased promoter activity. The most noticeable decrease was seen when a 102-bp sequence was deleted from the 983-bp construct. All the constructs with this 102-bp sequence showed higher promoter activity (relative luciferase activity >60; Fig. 2) than those without the 102-bp sequence. These results suggest that sequences in the vicinity of the PvuI site, especially the 102-bp sequence immediately upstream from the PvuI site, contain domains important for transcription activators.

Protein factors interacting with the 102-bp sequence in the vimentin promoter. We postulated that transcription factors interacting with the 102-bp promoter sequence are important for vimentin promoter activity. For identification of these protein factors, a series of EMSA experiments were performed using oligonucleotides and PCR fragments covering this promoter region. The oligonucleotide probe VP20 (Table 1), which resides at the 3′/H11032 end of the 102-bp sequence, formed a DNA-protein complex when it was incubated with nuclear extract proteins (see Fig. 4, lane WT). Search of the TFSEARCH database predicted two side-by-side binding sites in the VP20 sequence for GATA-1 and activator protein (AP)-4, respectively. To test these putative binding proteins, two mutant VP20 oligonucleotides were synthesized with destroyed consensus GATA-1 and AP-4 sites. However, EMSA results showed that wild-type and mutant oligonucleotides formed similar DNA-protein complexes. In addition, competition with unlabeled mutant VP20 oligonucleotides eliminated wild-type VP20 binding nearly completely (data not shown). These results suggest that formation of the DNA-protein complex is not dependent on the predicted GATA-1 and AP-4 sites.

Table 1. Wild-type and mutant oligonucleotides used in EMSA and affinity pull-down experiments

<table>
<thead>
<tr>
<th>Oligonucleotide</th>
<th>Sequence</th>
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<tbody>
<tr>
<td>VP20</td>
<td>AGGCGATGGGCCCAGCTGTA</td>
</tr>
<tr>
<td>VP20M3</td>
<td>AAAAGATGGGCCCAGCTGTA</td>
</tr>
<tr>
<td>VP20M4</td>
<td>AGGCGAACGCCCAGCTGTA</td>
</tr>
<tr>
<td>VP20M5</td>
<td>AGGCGATCAAAACCAGCTGTA</td>
</tr>
<tr>
<td>VP20M6</td>
<td>AGGCGATGGGAAACCAGCTGTA</td>
</tr>
<tr>
<td>VP20M7</td>
<td>AGGCGATGGGCCAAATGTA</td>
</tr>
<tr>
<td>VP20M8</td>
<td>AGGCGATGGGCCAGCAGAAA</td>
</tr>
<tr>
<td>AS2</td>
<td>GCTCCCCCCTCCCTCATCA</td>
</tr>
<tr>
<td>G6PD</td>
<td>GAGGGGCCCCCCTGAC</td>
</tr>
</tbody>
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G6PD, glucose 6-phosphate dehydrogenase. Underlined residues indicate mutations.

Fig. 1. Vimentin expression in lung epithelial cells. A: immunoblot showing levels of vimentin and cytokeratin-18 (K18) in lung epithelial cell lines (WI-26, Calu-3, A549, HAE, and H441). Presence of RT is indicated. B: RT-PCR amplification of vimentin and β-actin from HAE and H441 cells. Relative luciferase activities of the empty vector (basic) were adjusted to 1. Each experiment was performed 3 times.

Fig. 2. Activity of vimentin promoter in HAE cells. Left: a series of vimentin promoter-luciferase gene constructs. Solid lines indicate sequence regions in each construct. Restriction enzyme recognition sites are marked on the 1,463-bp vector at top. Arrow depicts 5′ end of the transcript. Solid boxes at the 3′ end represent the coding region of the vimentin gene. Arrowhead under the 1,463-bp construct marks location of the oligonucleotide VP20. Shaded area covers a 102-bp region important for the promoter activity. Right: relative luciferase activities aligned with each corresponding promoter construct at left. V, luciferase activity from the vector. Difference in luciferase activities between constructs 881 and 983 was analyzed by Student’s t-test (P = 0.002). Each promoter construct was tested 3 times.
PARP-1 binds to the vimentin promoter. To identify the protein interacting with VP20, we used nuclear protein pull-down experiments. Double-stranded VP20 oligonucleotide was biotinylated and coupled to magnetic beads (Dynabeads M-280 Streptavidin, Invitrogen). The VP20-coupled beads were then allowed to bind with HAE nuclear proteins by incubation with HAE nuclear extract. The bound proteins were eluted and examined by SDS-PAGE. SyproRuby stain of the gel showed a ~116-kDa band, which was absent in a parallel control experiment in which a different oligonucleotide (AS2, Table 1) was used (Fig. 3A). This band was excised and sent for protein identification by trypsin digestion followed by MALDI-MS analyses. A subsequent database search identified the 116-kDa band as PARP-1. The peptide coverage of the protein was 21.9% by mass and 22.1% by amino acids with 22 uniquely identified peptides.

To confirm that the VP20 binding protein is PARP-1, the proteins eluted from VP20-coupled Dynabeads were analyzed by immunoblotting. A single 116-kDa band was shown reacting with an anti-PARP-1 antibody (Fig. 3B). No band was seen in the control experiment in which the oligonucleotide AS2 was used. As a negative control, the same samples were examined in an immunoblot for transcription factor Sp1. Both samples were negative for Sp1, although the whole cell lysate was positive (data not shown). We also used a different vimentin-positive cell line, A549, in the nuclear protein pull-down experiment. As shown in the immunoblot in Fig. 3C, VP20-coupled beads pulled down PARP-1 (lane 1). As a control, the transcription factor Elk1 was examined on the same blot and was found in the whole cell lysate (lane 2), but not in proteins pulled down by VP20-coupled beads (lane 1). In addition to immunoblots, interaction between VP20 and PARP-1 was tested in EMSA using recombinant PARP-1. A DNA-protein complex was seen only in the reaction containing VP20. A mutant oligonucleotide, VP20M5, with a 3-bp change and a different oligonucleotide derived from the glucose 6-phosphate dehydrogenase promoter (Table 1) were used as negative control, and neither of these oligonucleotides bound recombinant PARP-1 (Fig. 3D). All these experiments suggest that PARP-1 binds to VP20 and that this binding is specific.

The PARP-1 binding domain in promoters is not well defined. In an effort to localize a defined region in VP20 for PARP-1 binding, a series of mutant oligonucleotides were synthesized and used for EMSA (Table 1). Except for M7, all mutants lost binding with the protein factor (Fig. 4). This suggests that a broad area in VP20 is involved in the interaction with the protein factor.

PARP-1 increases vimentin promoter activity in HAE cells. To assess the regulatory function of PARP-1 in vimentin transcription, promoter-luciferase reporter assay was used again. The role of PARP-1 was tested using a mutant 1,436-bp vimentin promoter construct with the 3-bp change used in VP20M5 (Table 1), which lost the ability to bind PARP-1 as...
H2O2 suppresses PARP-1 and vimentin expression. Similar to PARP-1, oxidative stress is also a regulator with complex roles in cancer growth. H2O2 is known to activate PARP-1 enzyme activity. In cancer cells, H2O2 not only induces EMT (28, 32), it also has been found to aid cancer drug therapy (31). Because vimentin is a protein marker of EMT, we were interested in the role of H2O2 in vimentin expression. We treated HAE cells with various concentrations of H2O2 and examined expression of PARP-1 and vimentin in immunoblots. Cytokeratin 18, another intermediate filament protein, was used to calibrate protein loading. We found that expression of PARP-1 and vimentin is suppressed with increasing concentrations of H2O2 (Fig. 6, A and B). We also examined whether H2O2 treatment resulted in cell death, which could contribute to the apparent reduction of vimentin and PARP-1 proteins. Acridine orange-propidium iodide assay results show that neither cell survival nor cell number is affected by ≤2 mM H2O2 (Fig. 6, C and D). Previous studies show that oxidative stress activates PARP-1 enzymatic activity in some cases (48) and induces PARP-1 cleavage in others (5). However, as previously reported (12), using a number of anti-PARP-1 antibodies, we did not see PARP-1 cleavage in HAE cells in immunoblots, even at 1 mM H2O2 (data not shown). Because PARP-1 directly binds and activates the vimentin promoter, it is likely that a reduced PARP-1 protein level mediates H2O2-induced inhibition of vimentin expression.

In addition to HAE cells, we also examined the effect of H2O2 in other vimentin-positive cell lines. Similar results were obtained in WI-26 and A549 cells (Fig. 7), suggesting that H2O2 may induce decreased protein levels of PARP-1 and vimentin in lung cancer cells. H2O2 induced a greater decrease in PARP-1 than in vimentin protein level. One explanation could be that H2O2 induces protein degradation of PARP-1 but not vimentin. Instead, the decline in vimentin protein level may be due to only decreased transcription and, thus, decreased new protein synthesis, whereas existing vimentin proteins might not be affected.

In the promoter assay, activity of the 1,463-bp vimentin promoter appears to be unaffected by increasing concentrations of H2O2 (cells without PARP-1 overexpression, Fig. 8). In contrast, in cells overexpressing PARP-1 (Fig. 8), vimentin promoter activity decreased steadily as H2O2 concentration increased, suggesting a role of PARP-1 in H2O2-induced suppression of the vimentin promoter. In Fig. 8, H2O2 did not inhibit vimentin promoter activity dose dependently. Because the relative value of luciferase activity is calculated as a ratio of vimentin promoter-driven firefly luciferase to HSV TK promoter-driven Renilla luciferase, if the both promoters were inhibited, the ratio would change little. This is exactly the case, as shown by inhibition of the HSV TK promoter by H2O2. It is not clear how H2O2 inhibited both promoters, but it could be mediated through endogenous PARP-1 in these cells or other unknown mechanisms. When the cells were cotransfected with the PARP-1 overexpression vector, inhibition of the vimentin promoter by H2O2 was much greater than inhibition of the TK promoter, resulting in a marked decline in the relative luciferase activity. These results suggest that PARP-1 may play a role in H2O2-induced inhibition of vimentin promoter activity.

DISCUSSION

Vimentin expression in lung cancer cells varies markedly (Fig. 1). This variation may signify the different stages of malignancy represented by these cells. In addition to its role as a cancer metastasis indicator, vimentin expression in alveolar epithelial cells is increased during the development of idiopathic pulmonary fibrosis (42), which increases risk of cancer (14). HAE is a lung cancer cell line that still expresses proteins of pulmonary epithelial cells such as the α-subunit of the epithelial sodium channel, surfactant protein A, and the CIC-2 chloride channel (by RT-PCR or immunoblotting; data not shown). Its high-level expression of vimentin, as well as very low-level

**Fig. 5. PARP-1 activates the vimentin promoter. A: overexpression of wild-type and mutant PARP-1 in HAE cells. Immunoblots show expression levels of wild-type and mutant PARP-1 in HAE cells transfected with vectors overexpressing wild-type PARP-1 (left) and a mutant PARP-1 with the COOH-terminal catalytic domain deleted (PARP-cat, right). Sp1 and endogenous PARP-1 are marked to indicate amounts of cell lysates loaded in the gels. B: promoter-reporter gene assay of the wild-type 1,463-bp vimentin promoter (1463) and the corresponding mutant promoter (1463M15) with 3-bp change identical to that of oligonucleotide VP20M5 (Table 1). In cotransfection experiments, vectors overexpressing wild-type PARP-1 and the COOH-terminal deletion mutant PARP-cat were used. **P < 0.05; ***P < 0.01 vs. 1463 (by Student’s t-test).**
expression of E-cadherin, suggests that HAE cells have undergone EMT. Examination of vimentin expression at the protein and mRNA levels, as well as vimentin promoter activity, allows one to conclude that the increased vimentin expression in HAE cells is due to an increased promoter activity (Fig. 1C). The vimentin promoter is regulated by many protein factors, including AP-1, PAE3, Sp1, Stat3, and ZBP-89 (8, 35, 44, 45). However, how the vimentin promoter is regulated in lung epithelial cells is not well understood. Initial study of different promoter regions found that a 102-bp sequence may contain an activation site for promoter activity in HAE cells (Fig. 2).

Fig. 6. H$_2$O$_2$ inhibits PARP-1 and vimentin expression in HAE cells. HAE cells were treated with various concentrations of H$_2$O$_2$ for 2 days. A: immunoblots showing expression of PARP-1, vimentin (VIM), and cytokeratin 18 in whole cell lysates. Band intensities of cytokeratin 18 were used to calibrate protein loading. B: relative band intensities of PARP-1 and vimentin plotted as the ratio of PARP-1 to cytokeratin 18 and the ratio of vimentin to cytokeratin 18, respectively. Relative band intensities at time 0 were set as 1. Experiments were repeated $\geq$ 3 times. All showed the same trends in PARP-1 and vimentin expression. C and D: acridine orange-propidium iodide assay to investigate whether H$_2$O$_2$ treatment results in cell death. Cells were treated with 0.25, 0.50, 0.75, and 1.00 mM H$_2$O$_2$ for 48 h, and changes in cell number ($10^5$ cells per 8-cm$^2$ surface area) and percent survival rate were plotted.

Fig. 7. H$_2$O$_2$ inhibits PARP-1 and vimentin expression in other lung epithelial cell lines. PARP-1 and vimentin expression in whole cell lysates of 2 vimentin-positive lung epithelial cell lines (WI-26 and A549) were examined by immunoblotting. Cells were treated for 2 days with 0.25 and 0.50 mM H$_2$O$_2$. Blots were incubated with PARP-1, vimentin (VIM), and cytokeratin 18 antibodies. Because WI-26 cells do not express cytokeratin 18, anti-β-actin antibody (ACT) was used to calibrate for protein loading. Each experiment was repeated twice.

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Fig. 8. PARP-1-mediated vimentin promoter activation is suppressed by H$_2$O$_2$. HAE cells were cotransfected with the 1,463-bp vimentin promoter-reporter gene vector and either the PARP-1 expression vector or its cloning vector. At 1 day after transfection, cells were treated with 0.25, 0.50, 0.75, and 1.00 mM H$_2$O$_2$ for 24 h. Luciferase activities were measured, and their relative values were calculated as the ratio of firefly luciferase activity to Renilla luciferase activity. Values of control assays were set at 100. Assays were repeated 3 times.
PARP-1 binds to the 3’ end of the 102-bp sequence (Fig. 3) and, therefore, may play a role in vimentin promoter activity. As a transcription factor, PARP-1 has been found to regulate a number of genes and interact with other transcription factors (9). It is not clear whether PARP-1 binds to any conserved DNA sequences. One reason for the lack of this information could be indirect binding of PARP-1 to DNA through other transcription factors, at least in some cases. In this study, a broad area in the oligonucleotide VP20 seems to be important for PARP-1 binding (Fig. 4), suggesting possible participation of other protein factors. However, EMSA experiments show that purified recombinant PARP-1 is able to bind directly with VP20 (Fig. 3D), supporting the notion that PARP-1 binds directly to DNA.

PARP-1 is well studied for its role in cell death. The prevailing concept of the role of PARP-1 is that DNA damage activates PARP-1 poly(ADP-ribosyl)ation activity, which consumes and depletes β-NAD+ and ATP in the cell. These events eventually cause energy failure and cell death. However, a second role of PARP-1 in transcription regulation has been increasingly recognized in recent years (9). Although they are not well understood, several mechanisms have been proposed. PARP-1-mediated poly(ADP-ribosyl)ation could disrupt the structure of chromatin, because histones can be highly poly-(ADP-ribosyl)ated (13). Transcription factors also could be modified and functionally altered by PARP-1-mediated poly-(ADP-ribosyl)ation (10, 30). Furthermore, PARP-1 may interact with promoter DNA directly or indirectly through other protein factors (27). It has been demonstrated using pharmacological inhibitors that PARP-1 enzymatic activity is not required in the activation of some genes (24). Using a PARP-1 mutant with the catalytic domain deleted, we show that the catalytic domain is not required for activation of the vimentin gene promoter (Fig. 5). This suggests that the DNA binding domain at the NH2 terminus of PARP-1 may mediate an interaction with vimentin promoter DNA.

The physiological significance of PARP-1-mediated transcription regulation is not yet quite understood. Evidence has emerged that regulation of PARP-1-mediated gene expression may play a role in inflammation- or oxidative stress-related disease conditions (25, 40). In this study, although mutation of the PARP-1 binding sequence in the vimentin promoter resulted in a 32% decline in promoter activity, the importance of PARP-1-mediated regulation of vimentin expression may be more profound under certain specific conditions. Oxidative stress is well known for its role in activating PARP-1 enzyme activity through increased DNA damage. In addition, oxidative stress in some of the most recent studies has been noted for its association with cancer development and EMT. In mouse mammary epithelial cells, treatment with matrix metalloproteinase-3 induces the expression of a splice variant of the small GTPase Rac-1, which subsequently induces EMT through increased cellular reactive oxygen species (ROS) (32). A positive correlation was also found between ROS levels and metastatic growth of cancer through the Rac-ROS pathway (18). Furthermore, ROS may play a role in transforming growth factor-β1- and 12-O-tetradecanoylphorbol-13-acetate-induced EMT (34, 43). These data suggest a potential role of oxidative stress in EMT. We show in this study that H2O2 treatment results in a decrease in PARP-1 and vimentin protein levels. Several mechanisms could be involved in this response. H2O2 induces DNA strand breaks, which activate PARP-1 enzymatic activity; H2O2 may also activate caspase-mediated cleavage of PARP-1, resulting in a 24-kDa protein containing the DNA-binding domain and an 89-kDa protein containing the catalytic domain (3). However, none of these mechanisms seems to play a role in this context, because the catalytic domain is not important in vimentin promoter activation (Fig. 5B) and PARP-1 is not cleaved by H2O2 in HAE cells (data not shown). Because PARP-1 binds to and activates the vimentin promoter, it is likely that the reduced vimentin level is associated with decreased PARP-1 protein. This is consistent with our finding that PARP-1-mediated vimentin promoter activity is suppressed by H2O2. Although oxidative stress is associated with EMT and compromises E-cadherin in some cell models (32, 34, 43), it was not clear whether oxidative stress-induced EMT involves increased expression of vimentin.

Our results in lung epithelial cells suggest that H2O2-induced EMT does involve increased vimentin expression. However, this response to oxidative stress could be cell type specific. Whether the response is the same in other cell systems remains to be investigated. It has been shown that H2O2 induces EMT in mouse mammary and rat kidney epithelial cells and that long-term oxidative stress induces invasive potential in mouse mammary epithelial cells (28, 32, 34). However, in the lung epithelial cell line H441, which does not express vimentin (Fig. 1), neither short-term nor long-term treatment with H2O2 (up to 1 wk) produced detectable vimentin expression (data not shown).

In summary, PARP-1 directly binds and activates the vimentin promoter in HAE cells independent of its catalytic domain. We show that PARP-1-activated vimentin expression is suppressed by H2O2 through its promoter. Other physiological significance of PARP-1 as a transcription regulator remains to be elucidated.

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