Human Polynucleotide Phosphorylase (hPNPase<sup>old-35</sup>): A Potential Link between Aging and Inflammation

Devanand Sarkar,¹ Irina V. Lebedeva,¹ Luni Emdad,¹ Dong-chul Kang,¹ Albert S. Baldwin, Jr.,⁴ and Paul B. Fisher¹,²,³

Departments of ¹Pathology, ²Neurosurgery, and ³Urology, Herbert Irving Comprehensive Cancer Center, Columbia University Medical Center, College of Physicians and Surgeons, New York, New York; and ⁴Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina

ABSTRACT

Chronic inflammation is a characteristic feature of aging, and the relationship between cellular senescence and inflammation, although extensively studied, is not well understood. An overlapping pathway screen identified human polynucleotide phosphorylase (hPNPase<sup>old-35</sup>), an evolutionary conserved 3',5'-exoribonuclease, as a gene up-regulated during both terminal differentiation and cellular senescence. Enhanced expression of hPNPase<sup>old-35</sup> via a replication-incompetent adenovirus (Ad.hPNPase<sup>old-35</sup>) in human melanoma cells and normal human melanocytes results in a characteristic senescence-like phenotype. Reactive oxygen species (ROS) play a key role in the induction of both in vitro and in vivo senescence. We now document that overexpression of hPNPase<sup>old-35</sup> results in increased production of ROS, leading to activation of the nuclear factor (NF)-κB pathway. Ad.hPNPase<sup>old-35</sup> infection promotes degradation of IκBα and nuclear translocation of NF-κB and markedly increases binding of the transcriptional activator p50/p65. The generation of ROS and activation of NF-κB by hPNPase<sup>old-35</sup> are prevented by treatment with a cell-permeable antioxidant, N-acetyl-l-cysteine. Infection with Ad.hPNPase<sup>old-35</sup> enhances the production of interleukin (IL)-6 and IL-8, two classical NF-κB-responsive cytokines, and this induction is inhibited by N-acetyl-l-cysteine. A cytokine array reveals that Ad.hPNPase<sup>old-35</sup> infection specifically induces the expression of proinflammatory cytokines, such as IL-6, IL-8, RANTES, and matrix metalloproteinase (MMP)-3. We hypothesize that hPNPase<sup>old-35</sup> might play a significant role in producing pathological changes associated with aging by generating proinflammatory cytokines via ROS and NF-κB. Understanding the relationship between hPNPase<sup>old-35</sup> and inflammation and aging provides a unique opportunity to mechanistically comprehend and potentially intervene in these physiologically important processes.

INTRODUCTION

Human polynucleotide phosphorylase (hPNPase<sup>old-35</sup>) was identified as a previously unknown gene, old-35, due to its up-regulation during cellular differentiation and senescence (1). hPNPase<sup>old-35</sup>, a 3',5'-exoribonuclease, is a predominantly type I interferon-inducible gene and is highly evolutionary conserved in plants, prokaryotes, and eukaryotes, with similar domain structures and functional properties in all species (1–3). Expression of hPNPase<sup>old-35</sup> is also augmented in senescent progenitor fibroblastoid cells in comparison with young fibroblasts (1). Overexpression of hPNPase<sup>old-35</sup> via a replication-incompetent adenovirus (Ad.hPNPase<sup>old-35</sup>) in HO-1 human melanoma cells and normal human melanocytes (NHuMel) produced a senescent phenotype characterized by growth arrest in the G<sub>1</sub> phase, increased senescence-associated β-galactosidase activity, decreased telomerase activity, and defined senescence-associated gene expression changes (4,5). These profound alterations induced by hPNPase<sup>old-35</sup> suggest an essential role in controlling senescence and differentiation, and it is likely that important intracellular mechanisms are modulated by hPNPase<sup>old-35</sup> to elicit these remarkable changes in cellular physiology.

Oxidative stress is a potential mediator of both in vitro replicative and premature senescence and in vivo aging (5). The free radical theory of aging, as proposed by Harman (6), states that endogenous reactive oxygen species (ROS) are generated in cells, resulting in a pattern of cumulative damage. Oxidative damage can be measured by formation of 8-oxo-2′-deoxyguanosine in DNA or free 8-oxoguanine base release by cells (7). Replicative senescence cells contain approximately 30% more 8-oxo-2′-deoxyguanosine in their DNA and produce four times more free 8-oxoguanine bases (7). Tissues from aged individuals or aged experimental animals accumulate oxidative damage in their DNA, protein, and lipids (7). Moreover, repeated subcytotoxic oxidative damage can induce premature senescence in multiple cell types such as fibroblasts, keratinocytes, melanocytes, or umbilical vascular endothelial cells (8), and treatment with a cell-permeable antioxidant or culturing cells in a reduced ambient oxygen permeable antioxidant or culturing cells in a reduced ambient oxygen permeable antioxidant or culturing cells in a reduced ambient oxygen peroxide (5). Although cytosolic enzymes such as NADPH oxidases contribute to the generation of ROS, the majority of intracellular ROS production is generated from mitochondria (5). Additionally, aged animals contain defective mitochondria and produce higher levels of ROS than their young counterparts (10).

A prominent mechanism by which ROS modulates diverse intracellular molecular processes is by regulating the activity of transcription factors, most notably nuclear factor (NF)-κB (11). As a corollary to increased ROS generation during the aging process, increased NF-κB DNA binding activity has been documented in multiple tissues of aged animals compared with young animals (12–15). In resting cells, NF-κB resides in the cytoplasm in an inactive form bound to an inhibitory protein known as IκB (16). On receiving a stimulus, such as ROS, IκB kinase is activated, which in turn phosphorylates IκB proteins, making them susceptible to ubiquitin-proteosome–mediated degradation (16). The destruction of IκB unMASKS the nuclear localization signal of NF-κB, leading to nuclear translocation and regulation of gene transcription by binding to the decameric motif GGGGN-NNYCC in the promoters of target genes (16). Presently, five mammalian NF-κB family members, NF-κB1 (p50/p105), NF-κB2 (p52/p100), p65 (RelA), RelB, and c-Rel, have been identified and cloned (16). The most abundant activated form of NF-κB is a heterodimer composed of a p50 and a p65 subunit that functions predominantly as a transcriptional activator.

hPNPase<sup>old-35</sup> induces a senescent phenotype and is up-regulated during senescence. The subcellular localization of hPNPase<sup>old-35</sup> is predominantly in the mitochondrial compartment (17). Based on these...
findings, we have now assessed whether hPNPase old-35 modulates ROS status in cells and, subsequently, the NF-κB signaling pathway. These experiments document that overexpression of hPNPase old-35 generates ROS that is responsible for activating the NF-κB pathway and its downstream genes, especially proinflammatory cytokines such as interleukin (IL)-6 and IL-8. Aged individuals are chronically incapacitated by inflammation, supporting the hypothesis that up-regulation of hPNPase old-35, which leads to the release of proinflammatory cytokines via ROS and NF-κB during senescence, might be an initiating factor mediating the pathogenesis associated with chronic inflammation.

MATERIALS AND METHODS

Cell Lines, Reagents, and Virus Infection Protocol. The human cervical carcinoma cell line HeLa and cultured Upstate previously (3). N-Acetyl-l-cysteine (NAC) and Tiron were obtained from Sigma (St. Louis, MO). The recombinant replication-incompetent adenovirus expressing hPNPase (Ad. hPNPase) was created in two steps as described previously and plaque-purified by standard procedures (1). Cells were infected with a multiplicity of infection (m.o.i.) of 1 to 50 plaque-forming units (pfu)/cell of Ad. vec (control replication-incompetent adenovirus) or Ad. hPNPase as described previously (18).

Transient Transfection and Luciferase Assay. Cells (5 × 104 cells per well in 12-well plates) were either uninfected or infected with either Ad. vec or Ad. hPNPase old-35 at a m.o.i. of 50 pfu/cell. Transient transfection was conducted 12 hours after infection using LipofectAMINE 2000 transfection reagent (Invitrogen, Carlsbad, CA) and 1.2 μg of plasmid DNA per well that included 1 μg of pGL3Basic, 3x-B-Luc, or 3x-Bmut-Luc plasmids (19) and 0.2 μg of β-galactosidase expression plasmid (pSV-β-gal; Promega, Madison, WI). For inhibition experiments, the cells were pretreated with different inhibitors for 2 hours before transfection. Luciferase assays were performed 48 hours after transfection using a Luciferase Reporter Gene Array Kit (Promega) according to the manufacturer’s protocol. The β-galactosidase activity was determined using the Galacto-Light Plus kit (Tropix, Bedford, MA). Luciferase activity was normalized by β-galactosidase activity, and the data from triplicate determinations were expressed as mean ± SD.

Generation of Anti-hPNPase old-35 Antibody. A COOH-terminal His-tagged hPNPase old-35 protein was produced in a baculovirus expression system (PharMingen, San Diego, CA) according to the manufacturer’s instructions. The protein was purified by Ni-NTA-agarose column and subsequently by ion-exchange chromatography. The purified protein was used to immunize chickens to generate anti- hPNPase old-35 antibody (Genetel Laboratories, Madison, WI).

Cell Fractionation and Electrophoretic Mobility Shift Assay. Cells were harvested, and the cytoplasm and nucleus were fractionated by the modified Schreiber’s method as described previously (20). Electrophoretic mobility shift assay (EMSA) using the nuclear extracts was performed as described previously (20). The sequences of the consensus and mutated NF-κB probes are 5'-AGTTGAGGCTTCTCCAGGC-3' and 5'-AGTTGAGGCTTCTCCAGGC-3,' respectively (Santa Cruz Biotechnology, Santa Cruz, CA). The antibodies used for supershift analysis were anti-p50, anti-p65, anti-p52, anti-p65, and anti-p52, respectively (rabbit polyclonal antibodies; Santa Cruz Biotechnology).

Preparation of Whole Cell Lysates and Western Blot Analysis. Whole cells were prepared, and Western blotting was performed as described previously (20). The primary antibodies used were anti-p50, anti-p52, 1:250 (rabbit polyclonal antibody; 1:250; Santa Cruz Biotechnology), anti-Elf1α (mouse monoclonal antibody; 1:1,000; Upstate Biotechnology, Lake Placid, NY), and anti-hPNPase old-35 (chicken; 1:10,000).

RNA Extraction and Reverse Transcription-Polymerase Chain Reaction. Total RNA was extracted from the cells using the RNeasy mini kit (Qiagen, Hilden, Germany) according to the manufacturer’s protocol. Two micrograms of total RNA were used for reverse transcription-polymerase chain reaction (RT-PCR) according to standard methods. The primers used were as follows: IL-6 sense, 5'-GGAGACTTTGTTGTTGTCG-3'; IL-6 antisense, 5'-GGACTTTGTTGTTGTCG-3'; IL-8 sense, 5'-GGACTTTGTTGTTGTCG-3'; IL-8 antisense, 5'-GGACTTTGTTGTTGTCG-3'; glyceraldehyde-3-phosphate dehydrogenase sense, 5'-ATGGGAGAGGTGAAGCTCCAGTGTC-3'; and glyceraldehyde-3-phosphate dehydrogenase antisense, 5'-GCTGTAGATCCTTGAAGCCTGTTGTC-3'.

RESULTS

Ad.hPNPase old-35 Infection Generates hPNPase old-35 Protein. The expression of hPNPase old-35 in HeLa cells after Ad.hPNPase old-35 infection was analyzed 2 days after infection by Western blot analysis. As shown in Fig. 1A, hPNPase old-35 expression was detected only in Ad.hPNPase old-35-infected cells. Subcellular localization revealed that Ad.hPNPase old-35 generated protein localizes in the mitochondria (data not shown), indicating that Ad.hPNPase old-35 infection generates functional protein.

Infection with Ad.hPNPase old-35 Induces Reactive Oxygen Species. The levels of intracellular ROS after Ad.hPNPase old-35 infection were determined using two dyes, DCFH-DA, which detects hydrogen peroxide, and HE, which detects superoxide or free hydroxyl radicals (21).

Infection with Ad.hPNPase old-35 induced both DCFH-DA and HE fluorescence in a time-dependent manner in comparison with Ad. vec infection (Fig. 1B and C). The generation of ROS by Ad.hPNPase old-35 was inhibited by treatment with a noncytotoxic dose (20 mmol/L) of a general antioxidant, NAC, which reduced the percentage of high ROS-containing cells from ~9% and ~15% to ~2% and ~3% at 24 and 36 hours, respectively (Fig. 1C).

Reactive Oxygen Species Mediates Activation of the Nuclear Factor-κB Pathway after Ad.hPNPase old-35 Infection. To test whether Ad.hPNPase old-35 infection activates the NF-κB pathway, HeLa cells were either uninfected or infected with Ad. vec or Ad.hPNPase old-35 and then transfected with either empty vector (pGL3Basic), 3x-B-Luc containing three tandem NF-κB binding sites upstream of the luciferase gene, or 3x-Bmut-Luc containing mutated NF-κB binding sites (19), and luciferase activity was analyzed. Cells transfected with either pGL3Basic or 3x-Bmut-Luc showed only basal luciferase activity under any experimental condition (Fig. 2A). In control and Ad. vec-infected cells, transfection of 3x-B-Luc increased basal activity over transfection of either pGL3Basic or 3x-Bmut-Luc, which is most likely a consequence of constitutive NF-κB DNA binding activity in HeLa cells. However, infection with Ad.hPNPase old-35 resulted in a 10- to 12-fold induction in relative luciferase activity in comparison with control or Ad. vec-infected cells (Fig. 2A). This activation could be effectively inhibited by treatment with increasing doses of either NAC or Tiron, indicating the involvement of ROS in Ad.hPNPase old-35-mediated NF-κB activation (Fig. 2B). NAC was much more potent than Tiron, and in additional studies, NAC was used.

The activation of NF-κB on Ad.hPNPase old-35 infection was further analyzed by EMSA using radiolabeled consensus NF-κB binding site as a probe and nuclear extracts from HeLa cells. As shown in Fig. 3A,
in independent experiments, each performed in triplicate.

The data represent the mean ± SD of three independent experiments, each performed in triplicate.

in uninfected cells and Ad.vec-infected cells, two shifted bands were observed at all time points. After Ad.\(hPNPase^{old-35}\) infection, the fast-migrating band started to disappear, with a substantial increase in the intensity of the slow migrating band. By 2 and 3 days after Ad.\(hPNPase^{old-35}\) infection, only the slow-migrating band was detected, and the intensity of the band was markedly higher in comparison with that in control and Ad.vec-infected cells. This change in DNA binding pattern could be observed with as little as 5 m.o.i. of Ad.\(hPNPase^{old-35}\) infection, and at 50 m.o.i., the intensity of the shifted band was substantially enhanced. All of the shifted bands were completely eliminated by a 100-fold excess of cold wild-type probe but not by cold mutated probe, indicating that these shifted bands are specific for NF-xB (Fig. 3B). The anti-p50 antibody supershifted both the slow- and fast-migrating bands, whereas anti-p65 antibody supershifted only the fast-migrating band. These findings demonstrate that under basal condition, both p50/p50 homodimers and p50/p65 heterodimers bind to the NF-xB probe. However, on Ad.\(hPNPase^{old-35}\) infection, the binding pattern changed, with the p50/p50 homodimer disappearing, and the binding of the p50/p65 heterodimer increasing markedly. These findings also indicate that \(hPNPase^{old-35}\) is a potent activator of NF-xB because it promotes increased binding of the potent transcriptional activator p50/p65. The specificity of NF-xB induction by Ad.\(hPNPase^{old-35}\) was demonstrated by the observation that infection with Ad.\(hPNPase^{old-35}\), but not infection with three unrelated adenoviruses, increased NF-xB DNA binding (Fig. 3C). Treatment with NAC significantly reduced the intensity of the shifted band induced by Ad.\(hPNPase^{old-35}\) infection (Fig. 3D), strengthening the conclusion that ROS acts as the second messenger to activate NF-xB after Ad.\(hPNPase^{old-35}\) infection.

The levels of p50 and p65 subunits of NF-xB and its inhibitor, IκBα, were analyzed in cytoplasmic and nuclear extracts after Ad.\(hPNPase^{old-35}\) infection. As shown in Fig. 3E, the levels of both p50 and p65 proteins began decreasing in the cytoplasmic extract of cells 2 days after Ad.\(hPNPase^{old-35}\) infection, whereas the level of p65 protein started increasing in the nuclear extract of Ad.\(hPNPase^{old-35}\)-infected cells 2 days after infection. These effects were not apparent in control or Ad.vec-infected cells (Fig. 3E and F), indicating that Ad.\(hPNPase^{old-35}\) infection resulted in translocation of p65 from the cytoplasm to the nucleus. The basal p50 protein level in the nucleus was quite high, and this level was not modulated substantially after Ad.\(hPNPase^{old-35}\) infec-
Fig. 3. Ad\textsubscript{hPNPase\textsuperscript{old-35}} infection increases NF-κB DNA binding and nuclear translocation by generating ROS. A. HeLa cells were infected as described in Fig. 1A or with Ad. vec at 50 m.o.i., or with Ad\textsubscript{hPNPase\textsuperscript{old-35}} at 1, 5, 10, or 50 m.o.i., and NF-κB DNA binding was analyzed in the nuclear extracts of the cells by EMSA at the indicated time points. B. HeLa cells were infected with Ad. vec or Ad\textsubscript{hPNPase\textsuperscript{old-35}} at 50 m.o.i., and NF-κB DNA binding was analyzed 2 days after infection. Cold WT, unlabeled consensus NF-κB probe; cold MUT, unlabeled mutated NF-κB probe. Supershift analysis was carried out with the indicated antibodies. *, supershifted band by anti-p50 antibody; **, supershifted band by anti-p65 antibody. C. HeLa cells were infected with the indicated adenoviruses at 50 m.o.i., and NF-κB DNA binding was analyzed 2 days after infection. D. HeLa cells were infected with Ad. vec or Ad\textsubscript{hPNPase\textsuperscript{old-35}} at 50 m.o.i. and treated with 20 mmol/L NAC or not treated. NF-κB DNA binding was analyzed 2 days after infection. E and F. HeLa cells were infected as described in Fig. 1A, and the expressions of the indicated proteins were analyzed in (E) cytoplasmic extract and (F) nuclear extract by Western blot analysis at the indicated time points. G. HeLa cells were infected as described in Fig. 1A and treated with 20 mmol/L NAC. The expressions of the indicated proteins were analyzed by Western blot analysis 2 days after infection.

**DISCUSSION**

We presently demonstrate that hPNPase\textsuperscript{old-35} activates the NF-κB pathway via the generation of ROS in HeLa cells. ROS is involved in the induction of a senescent phenotype characterized by irreversible growth arrest (8). Overexpression of hPNPase\textsuperscript{old-35} induces a senescence-like growth arrest and also generates ROS. Moreover, inhibition of ROS impedes the induction of NF-κB–responsive genes. The question naturally arises whether the inhibition of ROS can rescue HeLa cells from hPNPase\textsuperscript{old-35}–mediated growth inhibition. Our initial findings revealed that a noncytotoxic dose of NAC could not prevent Ad\textsubscript{hPNPase\textsuperscript{old-35}}–mediated growth inhibition.\textsuperscript{5} It is possible that gene expression changes, such as down-regulation of c-myc and up-regulation of p27\textsuperscript{KIP1} (4), resulting from Ad\textsubscript{hPNPase\textsuperscript{old-35}} infection might be primarily responsible for initiating growth arrest, whereas the generation of ROS might be involved in specifically regulating other cell signaling pathways such as NF-κB. Additional studies using normal human melanocytes or human diploid fibroblasts might provide additional insights to clarify these issues and help to define the function of hPNPase\textsuperscript{old-35}.
Contrasting results have been obtained regarding NF-κB binding activity during aging. No difference in NF-κB binding was observed between senescent and presenescent human fibroblasts as a function of in vitro replicative senescence (22). A reduced NF-κB activation in T cells from aged humans and mice has been reported previously (23). On the other hand, an increase in constitutive NF-κB DNA binding in older animals over young animals has been demonstrated in multiple studies (12–15). A gradual rise in ROS was evident in kidneys from Fischer rats from 6 to 24 months of age, and this increase correlated with an age-dependent augmentation in binding of p50/p65 NF-κB subunits, such as p50, IκBα degradation, p65 nuclear translocation, and elevated expression of cyclerenase-2, a NF-κB-responsive enzyme involved in inflammatory prostaglandin synthesis (13). Vascular smooth muscle cells from 18-month-old rats showed considerably higher p50/p65 NF-κB DNA binding than those from newborn rats, which correlated with increased expression of inducible nitric oxide synthase and intracellular adhesion molecule 1, two proinflammatory molecules, in old smooth muscle cells on inflammatory stimulation (14). A similar age-dependent elevation in NF-κB DNA binding has been reported in mouse and rat liver and heart and in rat brain (12, 15). In these tissues, an age-dependent rise in the levels of NF-κB subunits, such as p50, p52, and p65, could be observed. However, no change in the level of IκBα was detected. From these studies, it might be inferred that tissue-specific regulatory mechanisms may be involved in NF-κB activation during senescence. Although NF-κB activation requires degradation of IκBα, IκBα itself is a NF-κB-responsive gene (16, 24). During acute activation of NF-κB by TNF-α or related stimuli, there is an initial decrease in the cytoplasmic IκBα level followed by gradual restoration because of NF-κB-mediated transcription (24). Infection with Ad.hPNPaseold-35 resulted in a persistent decrease in the cytoplasmic IκBα level, indicating that even though NF-κB is activated by hPNPaseold-35, there might be an additional regulatory mechanism of IκBα transcription during senescence as compared with acute stimuli. A recent study has shown the lack of involvement of ROS in NF-κB activation by acute stimuli such as TNF-α (25). It is possible that in a state of chronic oxidative stress, such as senescence, ROS plays a role in activating NF-κB. Another intriguing observation is the selective induction of NF-κB-responsive genes by hPNPaseold-35 (Fig. 4), indicating that in addition to the primary transactivation of NF-κB by hPNPaseold-35, there might be a secondary level of regulation that targets the transactivation of specific NF-κB target genes.

What is the significance of induction of NF-κB-responsive genes by hPNPaseold-35 in the context of senescence? Ad.hPNPaseold-35 infection results in the up-regulation of proinflammatory cytokines via activation of NF-κB. By turning on proinflammatory cytokines, NF-κB functions as a central transcription factor for the development of chronic inflammatory diseases (26). Gene expression analysis by microarray in human hepatic stellate cells confirms that replicative senescence in these cells is associated with a pronounced inflammatory phenotype characterized by up-regulation of proinflammatory cytokines, including IL-6 and IL-8 (27). An aging-induced proinflammatory shift in cytokine expression profile has been observed in rat coronary arteries (28). Several studies have documented an increased
blood level of proinflammatory cytokines such as IL-1, IL-6, TNF-α, and IL-8 in aged individuals as compared with young individuals (29). The onset and course of a spectrum of age-associated diseases, such as cardiovascular disease, osteoporosis, arthritis, type 2 diabetes, Alzheimer’s disease, certain cancers, periodontal disease, frailty, and functional decline, might be associated with the production of proinflammatory cytokines (30, 31). Multiple studies have established an association between elevated levels of IL-6 and diseases of old age. IL-6 induces the production of C-reactive protein (CRP), an important risk factor for myocardial infarction (31). High concentrations of CRP predict the risk of future cardiovascular disease in apparently healthy men (31). IL-8 plays a crucial role in initiating atherosclerosis by recruiting monocytes/macrophages to the vessel wall, which promotes atherosclerotic lesions and plaque vulnerability (32). Elevated levels of IL-6 and CRP predict the development of type 2 diabetes in healthy women (33). In another study, elevated serum IL-6 levels predicted future disability in older adults especially by inducing muscle atrophy (34). IL-6 and CRP also play a pathogenic role in various diseases such as osteoporosis, arthritis, and congestive heart failure, all of which have increasing incidence with age (34). Moreover, increased serum levels of IL-6 and IL-8 have been detected in patients with chronic obstructive pulmonary diseases, and chemokines such as IL-8 and RANTES play important roles in the pathogenesis of these diseases (35, 36). Various inflammatory mediators, such as IL-1, TNF-α, IL-6, IL-8, RANTES, and MMP-3, are responsible for chronic inflammatory rheumatoid diseases such as osteoarthritis and rheumatoid arthritis, both of which occur during aging (37). The observation that the senescence-associated molecule hPNPase old-35 induces proinflammatory cytokines that are intimately involved in the development of aging-associated diseases suggests a potential involvement of hPNPase old-35 in these pathological processes.

In summary, we now document that hPNPase old-35 induces the expression of proinflammatory cytokines by generating ROS and activating NF-κB and hypothesize that these fundamental physiologic changes promoted by this gene might be essential contributors to aging-associated diseases. Additional studies are necessary to directly evaluate the role of hPNPase old-35 in chronic inflammatory diseases. These investigations will use cell culture and animal models and targeted inhibition experiments using dominant negative mutants and small interfering RNA to hPNPase old-35. Should a cause and effect relationship be observed between modulating hPNPase old-35 activity and delimiting inflammatory disease, such strategies might be amenable as therapeutic modalities for these debilitating and inevitable diseases of aging.

REFERENCES

Human Polynucleotide Phosphorylase (hPNPase\textsuperscript{old-35}): A Potential Link between Aging and Inflammation

Devanand Sarkar, Irina V. Lebedeva, Luni Emdad, et al.

*Cancer Res* 2004;64:7473-7478.

**Updated version**
Access the most recent version of this article at: [http://cancerres.aacrjournals.org/content/64/20/7473](http://cancerres.aacrjournals.org/content/64/20/7473)

**Cited articles**
This article cites 34 articles, 12 of which you can access for free at: [http://cancerres.aacrjournals.org/content/64/20/7473.full.html#ref-list-1](http://cancerres.aacrjournals.org/content/64/20/7473.full.html#ref-list-1)

**Citing articles**
This article has been cited by 6 HighWire-hosted articles. Access the articles at: [http://cancerres.aacrjournals.org/content/64/20/7473.full.html#related-urls](http://cancerres.aacrjournals.org/content/64/20/7473.full.html#related-urls)

**E-mail alerts**
Sign up to receive free email-alerts related to this article or journal.

**Reprints and Subscriptions**
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

**Permissions**
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.