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Inflammasome-Activated Caspase 7 Cleaves PARP1 to Enhance the Expression of a Subset of NF-κB Target Genes

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SUMMARY

Caspase 1 is part of the inflammasome, which is assembled upon pathogen recognition, while caspases 3 and/or 7 are mediators of apoptotic and non-apoptotic functions. PARP1 cleavage is a hallmark of apoptosis yet not essential, suggesting it has another physiological role. Here we show that after LPS stimulation, caspase 7 is activated by caspase 1, translocates to the nucleus, and cleaves PARP1 at the promoters of a subset of NF-κB target genes negatively regulated by PARP1. Mutating the PARP1 cleavage site D214 renders PARP1 uncleavable and inhibits PARP1 release from chromatin and chromatin decondensation, thereby restraining the expression of cleavage-dependent NF-κB target genes. These findings propose an apoptosis-independent regulatory role for caspase 7-mediated PARP1 cleavage in proinflammatory gene expression and provide insight into inflammasome signaling.

INTRODUCTION

The nuclear factor kappaB (NF-κB) signal transduction pathway is induced in response to a wide range of stimuli and regulates the cellular response to stress, inflammation, infections, and cytokines (Mankan et al., 2009). The transcription factor activity of NF-κB can be regulated by reversible posttranslational modification of NF-κB itself (e.g., acetylation), by the recruitment of cofactors (e.g., CBP/p300), or by the modification of chromatin structure (Lomvardas and Thanos, 2002; Natoli, 2006; Ramirez-Carrozzi et al., 2009).

Caspases are highly conserved cysteine-dependent aspartate-specific proteases. The human caspase family consists of 12 members that can be grouped into inflammatory and apoptotic caspases (Chowdhury et al., 2008). Caspase 1 is the prototypical member of the inflammatory caspases that mediate the maturation of inflammatory cytokines like proIL-1β and proIL-18 (Ghayur et al., 1997; Martinon et al., 2004; Thornberry et al., 1992). Caspase-1 is activated within a cytoplasmic multiprotein complex named the inflammasome, which consists of a receptor, an adaptor (e.g., ASC), and caspase 1 (Schroder and Tschopp, 2010). Genetic studies in mice suggest at least four inflammasomes with distinct receptors detecting different PAMPs (*p*athogen-*a*ssociated *m*olecular *p*atterns) and DAMPs (*d*anger-*a*ssociated *m*olecular *p*atterns).

Active caspase 3 and 7 cleave a large set of substrates, which ultimately results in apoptosis or necrosis (Kuida et al., 1996; Malireddi et al., 2010). Whereas caspase 3 knockout mice die prematurely (Kuida et al., 1996), caspase 7 knockout mice show no phenotypic abnormalities (Lakhani et al., 2006). Caspase 3/7 double-knockout mice suffer from early perinatal lethality (Lakhani et al., 2006). Accumulating evidence also suggests nonredundant roles and nonapoptotic functions for caspase 3 and caspase 7 in different cellular processes such as cell proliferation, cell-cycle regulation, and cell differentiation and inflammation (Algeciras-Schimnich et al., 2002; Denis et al., 1998; Lamkanfi et al., 2008).

Poly(ADP-ribose) polymerase 1 (PARP1, recently renamed ARTD1 [Hottiger et al., 2010]) is a nuclear chromatin-associated multifunctional enzyme found in most eukaryotes apart from yeast and catalyzes the polymerization of ADP-ribose units from donor NAD^+ molecules (Hassa et al., 2006; Kim et al., 2005). During apoptosis, PARP1 is cleaved by caspases 3 and 7, yielding two enzymatically inactive fragments (24 and 89 kDa) (D'Amours et al., 2001). Interestingly, PARP1 knockin mice, which harbor a mutated caspase cleavage site (D214N), develop normally, indicating that cleavage of PARP1 is not required during apoptosis (Pétrilli et al., 2004). This observation raises the fundamental question why such an elaborate mechanism for selective degradation of a subset of cellular proteins exists, when eventually all the cellular components need to be destroyed. Important regulatory functions other than apoptosis that are mediated by PARP1 cleavage could explain the development of this intricate mechanism.

Although historically studied in the context of genotoxic stress signaling, PARP1 has more recently been linked to the regulation of chromatin structure, transcription, and chromosome

organization (Kraus and Lis, 2003; Krishnakumar and Kraus, 2010). Based on the analysis of gene expression profiles in PARP1 knockout or siRNA treated cells, genes can be classified as PARP1-independent or PARP1-dependent (Frizzell et al., 2009), although the molecular basis that determines PARP1 dependency is not yet understood. Furthermore, PARP1-dependent genes can be regulated positively or negatively by PARP1 (Carrillo et al., 2004; Krishnakumar et al., 2008). Earlier studies from our laboratory provide evidence that in mouse lung fibroblasts (MLFs) the stimulus-dependent transcriptional activation of transiently transfected reporter plasmids containing the NF-κB sites of the *iNOS* and *MIP2* genes depends on PARP1 (Hassa et al., 2006; Hassa and Hottiger, 1999). Further studies revealed that the stimulus-induced gene expression of the *iNOS* (NF-κB) reporter plasmid containing the *nos-2* promoter was severely reduced in the presence of noncleavable (D214N) PARP1, while the presence of a cleavable PARP1 (WT or enzymatically inactive) was beneficial (Pétrilli et al., 2004). This observation was confirmed for endogenous *nos-2* and strongly suggests that PARP1 is most likely processed upon stimulation and that cleavage of PARP1 at D214 is beneficial for full transcriptional activation of certain NF-κB target genes. Here, we elucidate the cellular signaling mechanism that leads to PARP1 cleavage under inflammatory conditions and the consequence of this cleavage event for proinflammatory gene expression.

RESULTS

Induction of a Subset of NF-κB Target Genes Is Compromised in Peritoneal Macrophages Isolated from D214N PARP1 Mice

To investigate the regulatory function of PARP1 cleavage for NF-κB-dependent gene expression at the molecular level during inflammation, peritoneal macrophages expressing either WT or noncleavable D214N PARP1 were stimulated for 1 hr with LPS. Gene expression analysis by customized NF-κB microarrays (Jayne et al., 2009) identified ten genes that were less stimulated by LPS in D214N PARP1 cells as compared to WT cells (data not shown). Real-time RT-PCR revealed that the LPS-induced expression of *CSF2*, *IL-6*, and *LIF* was indeed dependent on PARP1 cleavage (reduced in D214N) (Figure 1A). IL-6 levels were also reduced at the protein level in LPS-induced D214N cells (Figure S1A). Interestingly, the three genes were highly expressed in *PARP1* −/− macrophages, indicating a repressory function of PARP1 for these genes (Carrillo et al., 2004) (data not shown). IP-10 was also negatively regulated by PARP1 (PARP1-dependent), but in contrast to the other three genes comparably induced in WT and D214N PARP1 cells (Figure 1A) and was therefore included as a control gene, which is not expressed in a PARP1 cleavage-dependent manner. Together, these data show that only a subset of PARP1-dependent genes was additionally regulated by PARP1 cleavage.

PARP1 Cleavage at D214 Regulates the LPS-Induced Expression of NF-κB Target Genes Also in Human THP1 Cells

To further investigate the physiological relevance of LPS-induced PARP1 cleavage, we expressed WT or noncleavable D214N PARP1 in human THP1 cells depleted of endogenous PARP1 (Figure S1B). LPS stimulation (1 hr) of THP1 cells complemented with noncleavable D214N PARP1 (as compared to THP1 cells complemented with WT *PARP1*) resulted in significantly reduced *IL-6* gene induction, while *IP-10* induction was not affected (Figure 1B), and thus implied that PARP1 cleavage is important for the transcriptional activation of *IL-6* and confirmed the results obtained with primary cells.

Since PARP1 cleavage is considered a hallmark of apoptosis, we tested whether LPS treatment of THP1 cells would induce cell death under the tested conditions by two independent methods. While the control treatment with camptothecin (CPT) induced a significant increase of dead cells, LPS stimulation did not induce any detectable increase in cell death for the indicated time period as measured by staining with ethidium homodimer and calcein or LDH release (Figures 1C and 1D). Moreover, treatment of complemented THP1 cells with CPT induced an 89 kDa cleavage fragment in WT-complemented cells, but not in cells expressing D214N PARP1, confirming that the mutant D214N PARP1 was indeed protected from caspase cleavage (Figure 1E).

The PARP1 D214N Mutation Does Not Affect NF-κB Function or Biochemical Properties of PARP1

The observation that *CSF2* and *IL-6* induction were both abolished upon knockdown of the large NF-κB subunit p65 (Figures S1C and S1D) suggested that stimulus-induced expression of these genes is mainly dependent on p65 signaling. Therefore, we performed ChIP experiments with an antibody against p65 to investigate whether the reduced gene induction in D214N PARP1 cells was due to hampered recruitment of p65 to target DNA sequences. p65 recruitment to the promoters of *CSF2*, *IL-6*, and *IP-10*, but not to the promoter of the control gene *Prolactin*, occurred as soon as 30 min after LPS stimulation of peritoneal macrophages isolated from WT and D214N animals (Figure 2A). After 1 hr, p65 dissociated from the *IL-6* and *IP-10* promoters and exhibited a comparable distribution in WT and D214N cells (Figure 2A). These results clearly suggested that the differential expression of the studied NF-κB target genes was not due to differential p65 recruitment.

In order to exclude that the above-described expression changes were due to altered biochemical properties of D214N PARP1, additional analyses were performed. The enzymatic activity of WT and D214N PARP1 was induced by DNA to the same extent in vitro (Figure 2B). Since the stimulation by DNA is known to induce a conformational change that brings the N-terminal DNA-binding domain to the C-terminal catalytic domain of PARP1 proteins (Messner et al., 2010), the D214N mutation is unlikely to alter the overall configuration of PARP1. We have previously described that PARP1 can bind and be acetylated by p300 (Hassa et al., 2005). Acetylation of PARP1 by p300 revealed that D214N and WT PARP1 can be acetylated in vitro to the same extent (Figure 2C), implicating that complex formation with other proteins was not affected. Complex formation between p65 and PARP1 was also not affected by the D214N mutation in vivo as well as in vitro (Figures 2D and 2E).

These results furthermore implied that the reduced induction of gene expression observed in D214N cells was neither due to