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DNA repair Ku proteins in gastric cancer cells and pancreatic acinar cells

Minireview Article

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Summary. The DNA repair protein Ku acts as a heterodimer of Ku70 and Ku80 that binds to the DNA ends, nicks, or single-to-double-strand transition. It has a crucial role for DNA double-strand-break repair. Either Ku70 or Ku80 itself may have a unique function that is independent of the other Ku subunit. In this review, the role of Ku on cell proliferation and apoptosis will be discussed. Ku acts as a regulator of transcription by interacting with the recombination signal binding protein Jκ and the NF-κB p50 homodimer to up-regulate p50 expression, which may regulate the proliferation of gastric cancer cells. Both Ku70 and Ku80 expressions are mediated by constitutively activated NF-κB and constitutively expressed cyclooxygenase-2 in gastric cancer cells, which may be related to gastric cell proliferation and carcinogenesis. In addition, nuclear loss of Ku may underlie the mechanism of apoptosis in pancreatic acinar cells after oxidative stress.

Keywords: DNA repair Ku protein – NF-κB – Cylooxygenase-2 – Apoptosis – Gastric cancer cells – Pancreatic acinar cells

Introduction

The Ku70 (70-kDa) and Ku80 (80-kDa) proteins are DNA-binding regulatory subunits of DNA-dependent protein kinase (DNA-PK), which are composed of a 470-kDa catalytic subunit (DNA-PKcs) and Ku proteins (Featherstone and Jackson, 1999; Bliss and Lane, 1997). The Ku70 and Ku80 proteins act as the regulatory parts of the DNA-PK and initiate the repair process of DNA double-strand breaks, which produce DNA fragmentation, by activating DNA-PK after binding to the DNA double-strand breaks (Finnie et al., 1995). In addition to the regulatory function of the Ku proteins in DNA-PK, heterodimers of both Ku70 and Ku80 also have independent DNA repair functions. These include single-stranded DNA-dependent ATPase activity and the binding and repair of broken single-stranded DNA, single-stranded nicks, gaps in DNA, and single-strand-to-double-strand transitions in DNA (Featherstone and Jackson, 1999; Bliss and Lane, 1997). Besides this, Ku70 and Ku80 themselves may each have unique functions including cell proliferation, which are independent of the other Ku subunit. It was reported that a Ku70 and Ku80 deficiency but not a DNA-PKcs deficiency resulted in a dramatic increase in cell apoptosis. Here the role of Ku on cell proliferation and apoptosis in gastric cancer cells and pancreatic acinar cells will be discussed.

Recombination signal binding protein Jκ (RBP-Jκ) is a DNA-binding protein, which participates in the control of both cytokine and NF-κB p52 protein expression (Krauer et al., 1998). Several genes, known to be repressed or activated by RBP-Jκ, possess a κB element containing overlapping NF-κB and RBP-Jκ binding elements in their promoter regions (Oswald et al., 1998). NF-κB subunit expression, as well as NF-κB activity, is regulated by the Ku expression level in certain cells. Therefore, Ku may act as a regulator of transcription by interacting with the recombination signal binding protein Jκ and the NF-κB p50 homodimer to up-regulate p50 expression, which may regulate cell proliferation of gastric cancer cells.

Prostaglandin (PGE_2), produced by cyclooxygenase-2 (COX-2), decreased cell death and regulated cell proliferation in cultured tumor cells (Raz, 2002). COX-2 inhibition by a selective COX-2 inhibitor NS-398 induced apoptosis with a lower Bcl-2 protein level in human prostate cancer cells (Liu et al., 1998). Bcl-2 and COX-2 play a role in the early genesis/progression of a gastric carcinoma

(Gao et al., 2000). Therefore, PGE_2 might be involved in cell growth and proliferation and enhance the tumorigenic potential in some cancer cells (Raz, 2002). Both Ku70 and Ku80 expressions are higher in aggressive breast tumors than those in normal tissues (Pucci et al., 2001). These studies suggest a possible relation of the PGE_2 by COX-2, the levels of Ku70 and Ku80, and cell proliferation in cancer cells.

The severe DNA damage, which is beyond the capacity of the DNA repair proteins to correct, triggers apoptosis. Apoptosis linked to oxidative DNA damage has been reported in pancreatitis (Kaiser et al., 1995; Sandoval et al., 1996). It has been suggested that oxidative stress may induce a decrease in the Ku70 and Ku80 levels and cause apoptosis in pancreatic acinar cells (Song et al., 2003). Oxidative injury caused by ischemia/reperfusion in the rabbit spinal cord induces reversible neurological deficits with increased Ku-DNA binding activity, which is an indicator of DNA-PK activation, whereas severe ischemia/reperfusion causes permanent deficits that are accompanied by a decrease in the Ku-DNA binding activity (Shackelford et al., 1999). The studies suggest that Ku may have a defensive role against oxidative injury.

In this review, I will discuss: 1) the interaction of Ku with RBP-Jκ and NF-κB p50 as a positive regulator for p50 expression in gastric cancer AGS cells; 2) gastric cell hyperproliferation mediated by Ku70 and Ku80 in a COX-2-dependent mechanism; and 3) the role of Ku in the mechanism of apoptosis in pancreatic acinar cells after oxidative stress.

Ku-recombination signaling binding protein Jκ (RBP-Jκ) complex and NF-κB in proliferation of gastric cancer cells

NF-κB is a transcription factor that regulates the wide variety of genes that respond to immune or inflammatory signals (Baeuerle and Baltimore, 1996). NF-κB is a member of the Rel family, which includes p50 (NF-κB1), p52 (NF-κB2), Rel A (p65), c-Rel and Rel B (Siebenlist et al., 1994). In resting cells, NF-κB is localized in the cytoplasm as a hetero- or homodimer, which is noncovalently associated with cytoplasmic inhibitory proteins, including IκBα. Upon stimulation by a variety of pathogenic inducers such as viruses, mitogens, bacteria, agents providing oxygen radicals, and inflammatory cytokines, IκBα is phosphorylated, ubiquitinated and degraded in the cytoplasm, and the NF-κB complex migrates into the nucleus and binds the DNA recognition sites in the regulatory regions of the target genes (Thanos and Maniatis, 1995).

Some stimuli, including phorbol ester, TNF, and platelet activating factor (PAF), induce p50 mRNA expression (Meyer et al., 1991). The p50 expressions in lung cancer tissues are higher than those in counterpart normal tissues, suggesting that they may be related to tumor or cancer development (Mukhopadhyay et al., 1995). It has been reported that p50 induction is partially mediated by members of the NF-κB family by binding the κB element in the p50 promoter (Schooley et al., 2003). However, comparatively little is known about the factors and signal transduction pathways which contribute to the regulation of p50 expression.

Recombination signal binding protein Jκ (RBP-Jκ) is a DNA-binding protein, which regulates both cytokine and NF-κB p52 protein expression (Krauer et al., 1998; Oswald et al., 1998). Several genes, known to be repressed or activated by RBP-Jκ, possess a κB element containing both NF-κB and RBP-Jκ binding elements in their promoter regions (Oswald et al., 1998). The studies suggest that there may be a possible interplay between NF-κB and RBP-Jκ for binding to the κB element in the p50 promoter.

Ku, as a heterodimer of Ku70 and Ku80, is the regulatory DNA-binding region of the DNA-dependent protein kinase (DNA-PK), which has been implicated in several nuclear processes, including the repair of broken DNA double strands and V(D)J recombination (Sawaoka et al., 1998). Ku possesses a strong affinity for DNA ends and peculiar DNA structures such as nicks, gaps and hairpins in a sequence-specific manner (Pedley et al., 1998). Putative Ku-specific binding elements have been located in variety of genes such as c-myc, collagen III and HIV-1 (Pedley et al., 1998; Jeanson and Mouscadet, 2002). It has been suggested that Ku is involved in the positive or the negative regulation of these genes. Um et al. (2001) showed that over-expression of Ku increases nuclear NF-κB activity in Rat-1 fibroblast. Therefore, NF-κB subunit expression, as well as NF-κB activity, may be regulated by the Ku expression level in certain cells.

Previously, we found that inhibition of Ku activity by transfection with the C-terminal Ku80 expression gene suppressed the expression of p50 but not of p65 in gastric AGS cancer cells (Lim et al., 2004). Transfection of the C-terminal Ku80 expression gene also decreased nuclear NF-κB activity. We then demonstrated that RBP-Jκ is involved in p50 expression and p50 expression is mediated by the κB element in the p50 promoter. RBP-Jκ binds to several κB elements in the promoter of the NF-κB inducible gene and regulates gene expression.

RBP-Jκ-mediated expression includes its association with several proteins, including SMART, HDAC-1 and SHARP (Oswald et al., 2002). The evidence demonstrates a novel role of Ku as an RBP-Jκ interacting protein. Furthermore, RBP-Jκ binds to a 5′ flanking sequence of the κB element (TGGGGG) in the p50 promoter, which overlaps the p50 binding site. RBP-Jκ has been known to bind to an essential core consensus DNA sequence, TGGGAA (Ling et al., 1994). However, the p50 promoter has a poor consensus sequence, TGGGGG, in which RBP-Jκ nevertheless binds. Promoter regions in a number of other genes contain slightly modified RBP-Jκ binding sites. For instance, RBP-Jκ binds to a poor consensus sequence, GCTGAGAT, in cyclin D1 promoter (Iso et al., 2002). Therefore, the RBP-Jκ-binding sequence is not strict and the variant RBP-Jκ binding could occur.

Ku is known to bind sequences specifically to DNA as well as to DNA ends. It is evident that Ku binds specifically to the downstream of the κB element in the p50 promoter. Our previous study showed that the Ku binding sequence is GGTTC (Lim et al., 2004). In comparison with the NRE1 element previously reported as the Ku binding sequence, the Ku binding sequence we found is similar to the NRE1 sequence. Since the Ku binding core consensus sequence is not defined, further studies concerning the Ku binding sequence should be performed. All these results clearly suggest that all of Ku, RBP-Jκ and NF-κB positively regulate p50 expression in gastric AGS cancer cells. Ku acts as a regulator of transcription by interacting with RBP-Jκ and the NF-κB p50 homodimer to up-regulate p50 expression in gastric AGS cancer cells.

Mounting evidence shows that p50 expression is related to tumorigenesis and carcinogenesis of certain types of the cells (Mukhopadhyay et al., 1995), and p50 and Ku antigens are involved in cell growth and proliferation (Lim et al., 2002; Sadji et al., 2000; Li et al., 2002). Previously we showed that the gastric cell hyperproliferation associated with carcinogenesis might be associated with both high expression and high nuclear levels of Ku70 and Ku80 in a COX-2-dependent mechanism, which is mediated by NF-κB activation in gastric cancer AGS cells (Lim et al., 2002). We infer that Ku as well as NF-κB, induced by the signals which are associated with cell growth and proliferation, may increase the p50 expression and nuclear NF-κB activity. Further studies should be carried out to determine whether Ku antigen activity and p50 expression are pivotal in molecular regulation of cell growth and proliferation.

Ku, NF-κB, and COX-2 in proliferation of gastric cancer cells

NF-κB is constitutively activated in B-cell lymphoma, breast and gastric cancer cells (Wu et al., 1996; Sovak et al., 1999; Kim et al., 1999). Cell proliferation and tumorigenesis involve the constitutive induction of NF-κB activation. The NF-κB target genes have been implicated in the prevention of cell death by regulating the expression of genes such as the tumor necrosis factor, receptor-associated factors, TRAF1 and TRAF2, the inhibitor of the apoptosis proteins, c-IAP1 and c-IAP2, and Bcl-X_L and Bcl-2 (Barkett and Gilmore, 1999). COX-2 is constitutively expressed in some cancers (Battu et al., 1998; Lim et al., 2001) and is related to cell proliferation (Molina et al., 1999). NF-κB was constitutively activated in human gastric carcinoma tissue as opposed to the adjacent normal epithelial cells (Sasaki et al., 2001). Premalignant and malignant gastric lesions exhibit strong COX-2 expression, which is not observed in normal lesions (Sung et al., 2000; Gao et al., 2000). A previous study demonstrated that constitutively expressed COX-2, which is mediated by constitutive NF-κB, regulates cell proliferation in AGS gastric cancer cells (Lim et al., 2001). This suggests that constitutive COX-2 expression via constitutive NF-κB may be a principal mechanism for gastric carcinogenesis and tumorigenesis. Several studies have shown that inhibiting the expression of either Ku70 or Ku80 results in the inhibition of cell growth and the induction of apoptosis (Nussenzweig et al., 1996; Gu et al., 1997).

Our previous study showed that the AGS cells with a low level of constitutive NF-κB had a lower expression level of Ku70 and Ku80. This finding contrasts with the report by Um et al. (2001), where PC12-NF-κB cells overexpressing both p50 and p65 subunits of NF-κB exhibited an increase in Ku70 and Ku80 expression compared with the parental PC-12 cells. COX-2 inhibitors such as indomethacin and NS-398 were found to suppress Ku70 and Ku80 expression in AGS cells. PGE_2, a COX-2 product, enhanced the Ku70 and Ku80 expression levels in the cells with a low constitutive NF-κB level. These results suggest that Ku70 and Ku80 expression may be regulated by the COX-2 and COX-2 product, prostaglandins, via a NF-κB-dependent mechanism in AGS cells.

Prostaglandins exerted their biological action via specific receptors (EP-1, -2, -3 and -4) (Narumiya et al., 1999). PGE_2 transactivated the epidermal growth factor receptors (EGFR), which then triggered mitogenic signaling (Malecka-Panas et al., 1997). Efficient DNA repair in actively growing cells requires growth factor signaling

(Mendelsohn and Fan, 1997; Haimovitz-Friedman et al., 1991). EGFR-mediated signaling is associated with mitogenesis and cell proliferation (Aaronson, 1991; Kumar and Mendelsohn, 1991). Since EGFR signaling requires the maintenance of a nuclear level of DNA-PK and its regulatory heterodimeric complex, Ku70 and Ku80, in mammalian cells (Bandyopadhyay et al., 1998), the role of Ku70 and Ku80 on cell proliferation and growth is postulated. In addition, a heterodimer of Ku70 and Ku80 is a regulatory subunit of DNA-PK that phosphorylates many proteins, including transcription factors such as c-Jun, c-Fos, c-Myc and many more. They appear to be multifunctional proteins, which are implicated in cellular processes, including DNA replication, transcriptional regulation, and controlling the G2 and M phases of the cell cycle (Tuteja and Tuteja, 2000). Therefore, Ku70 and Ku80 may be involved in cell proliferation by regulating the cell cycle-associated proteins or growth-related gene expression.

We previously demonstrated that the inhibition of the Ku DNA-end-binding activity by the transfection of C-terminal Ku80 (427–732) expression gene resulted in the suppression of cell proliferation in AGS cells (Lim et al., 2002). Several reports showed that inactivating Ku80 or Ku70 reduced the expression of the other Ku subunit (Ku70 or Ku80), and then inhibited the Ku DNA-end-binding activity as well as the DNA-PK activity in either the Ku70- or Ku80-deficient cells (Nussenzweig et al., 1996; Li et al., 2002; Gu et al., 1997). From these results, it is suggested that a disruption of either of the Ku subunits would reduce the Ku DNA-end-binding activity, which then inhibits the functional role of the Ku proteins. Therefore, the inhibition of the cell growth caused by a reduction in the nuclear level of Ku70 and Ku80 may be related to the loss of the Ku DNA-end-binding activity. Null knockout mice for DNA-PKcs did not exhibit growth retardation, whereas growth retardation was observed in either Ku70 or Ku80 knockout mice (Nussenzweig et al., 1996; Gu et al., 1997). This suggests that Ku70 and Ku80 are associated with growth regulation independent of the function of DNA-PK. Li et al. (2002) demonstrated that Ku80 inactivation resulted in the induction of the tumor suppressor protein p53, which contributed to the inhibition of cell growth.

Inhibition of PGE_2 production by sulindac (Taylor et al., 2000), had a marked inhibitory effect on the development of colon tumors in mice (Moorghen et al., 1998). COX-2 inhibition by NS-398 induced apoptosis in human prostate cancer cells (Liu et al., 1998). Therefore, PGE_2 might be involved in cell growth and proliferation and enhance the tumorigenic potential in some cancer cells. In B cell chronic lymphocytic leukemia (B-CLL), the level of anti-apoptotic Bcl-2 showed a positive correlation with the Ku80 level (Klein et al., 2000). Both Ku70 and Ku80 expressions are higher in aggressive breast tumors compared with those in normal tissues (Pucci et al., 2001). These studies suggest a possible relation of the PGE_2 by COX-2, Ku70 and Ku80, and cell proliferation in cancer cells. It is suggested that the gastric cell hyperproliferation associated with carcinogenesis might be associated with both high expression and high nuclear levels of Ku70 and Ku80 in a COX-2-dependent mechanism, which is mediated by NF-κB activation in gastric cancer cells. Further studies should focus on the action mechanism of COX-2 and its products on Ku70 and Ku80 expressions, and the possible mechanism and mediator(s) that induce cell proliferation by Ku70 and Ku80 in gastric cancer cells.

Ku and oxidative stress in apoptosis of pancreatic acinar cells

The relationship between reactive oxygen species (ROS) and apoptosis in the pathophysiology of acute pancreatitis has been reported (Kerr et al., 1972; Carson and Ribeiro, 1993). In 38 chronic pancreatitis patients, the distribution of caspase-1 expression was correlated with the extent of apoptosis of acinar cells in pancreatic tissues (Ramadani et al., 2001). These studies indicated the possibility that the development of apoptotic cell death caused by ROS may reflect the severity of pancreatitis, which might have substantial clinical value.

Oxidative damage to the nucleic acids can produce adducts of the base and sugar residues, which may lead to the generation of single-strand breaks. Less frequently, oxidation can cause cross-links to other molecules and double-strand breaks (Beckman and Ames, 1998). Several studies have shown that the inhibition of either Ku70 or Ku80 expression resulted in the induction of apoptosis in human promyelocytic leukemia HL-60 cells and activated human peripheral blood lymphocytes (Ajmani et al., 1995). There is some evidence suggesting the involvement of caspase-3 activation in H_2O_2-induced apoptosis in HL-60 cells (Matsura et al., 1999; Mizutani et al., 2002) and human neuroblastoma cells (Jang et al., 2002). They suggested H_2O_2 as a second messenger of the death signal in some diseases linked to oxidative stress stimuli. Both cell death and PARP (poly [ADP-ribose] polymerase) cleavage were prevented either by the caspase-3 inhibitor (Matsura et al., 1999; Velez-Pardo et al., 2002) or by a known antioxidant, glutathione (Emannele et al., 2002). In contrast, high concentrations of H_2O_2 (above 300 nmol/ml)

suppressed both the activation and the activity of the caspases, possibly by modulating the redox status of the cells and the oxidation of the cysteine residues in the caspases. In our previous study (Song et al., 2003), the reduction in nuclear Ku proteins of pancreatic acinar AR42J cells undergoing apoptosis was blocked by the treatment with the caspase-3 inhibitor. The caspase-3 inhibitor prevented the loss of nuclear Ku70 and Ku80 in apoptotic cells. These results suggest that the caspase cascade during apoptosis may involve the degradation of Ku70 and Ku80 of pancreatic acinar cells. Therefore, after oxidative stress, the reduction in Ku might be involved downstream from the caspase-activating apoptotic pathway. In drug-induced apoptosis in various cells, an increase in ubiquitinated Ku70 was observed (Gama et al., 2006). The results demonstrate that the ubiquitin-proteosome proteolytic pathway plays a role in decreasing Ku70 levels in apoptotic cells. Bacsi et al. (2005) reported that there was no change in the protein levels of DNA-PKcs, Ku70, or Ku80, but that there was a decrease in DNA-PK activity, suggesting that oxidative stress affects post-translational modification and assembly of DNA-PK complex at DNA double-strand breaks, and thereby repair of DNA double-strand breaks.

There are studies on the subcellular localization of Ku70 and Ku80 (Koike et al., 1999a; Morio et al., 1999), demonstrating that the control mechanism for subcellular localization of Ku70 and Ku80 plays a key role in regulating the physiological function of Ku *in vivo*. Each Ku subunit can translocate to the nucleus not only through its own nuclear localization signal (NLS), but also through heterodimerization with each other (Koike et al., 1999b). The Ku70 NLS and the Ku80 NLS are mediated to target to the nuclear rim by two components of the nuclear pore-targeting complex, importin α and importin β (Koike et al., 1999a). The active nuclear transporter, importin α, has an essential role for the nuclear transportation of the apoptotic signaling molecules including the caspases (Yasuhara et al., 1997). Since Ku70 and the caspases share the same nuclear transporter, Ku70 may be cleaved by the caspases in the nucleus during apoptosis. This possibility is supported by studies showing that activated caspase-3 was observed in the nucleus during apoptosis (Mandal et al., 1999) and that both the DNA-PKcs and the poly (ADP-ribose) polymerase (PARP) are substrates for the caspase-3-like activities after ischemia/reperfusion (Shackelford et al., 1999). In addition, the well-known nucleoporins Nup153, RanBP2, Nup214 and Tpr, which bind the soluble components of the nuclear transport machinery such as the importin family and the small GTPase Ran (Saitoh et al., 1996), are cleaved by caspases during apoptosis (Ferrando-May et al., 2001). Nucleoporin cleavage may affect the nucleocytoplasmic transport in the cells during apoptosis even though nuclear transport factors Ran, importin α and importin β are not proteolytically processed (Ferrando-May et al., 2001). We suggested that the inhibition of the nuclear translocation of the Ku proteins by oxidative stress might be another possible explanation for the loss of nuclear Ku70 and Ku80 and the slight increases in the cytoplasmic Ku proteins in pancreatic acinar AR42J cells undergoing apoptosis (Song et al., 2003). Since the Ku binding to importin family was decreased and nuclear Ku proteins were degraded by oxidative stress in our previous study, the significant loss of the nuclear Ku proteins was shown in the cells exposed to oxidative stress, resulting in the loss of defense against oxidative DNA damage and apoptosis in pancreatic acinar cells.

The oxidative stress-induced apoptosis may be mediated by the activated caspase-3, which degrades the DNA repair protein Ku70 and Ku80, and decreases Ku binding to nuclear transporter importin α and importin β, resulting in the reduced nuclear Ku proteins in pancreatic acinar cells. The nuclear loss of Ku70 and Ku80 may cause the loss of the defense against oxidative DNA damage, which underlies the mechanism of apoptotic cell death in pancreatic acinar cells after oxidative stress (Fig. 1).

In summary, the expression of Ku is mediated by NF-κB in gastric cancer AGS cells. Inhibition of Ku activity decreases both p50 expression and nuclear NF-κB activity in gastric cancer cells. Ku interacts with RBP-Jκ, a DNA-binding protein. Additionally, Ku, RBP-Jκ and p50

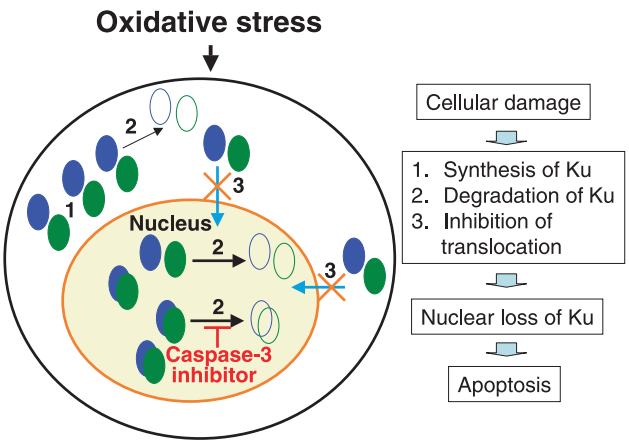

Fig. 1. The postulated mechanism of oxidative stress-induced apoptosis in pancreatic acinar cells. Nuclear decrease in Ku70 (blue circle) and Ku80 (green circle) may result from degradation of Ku proteins (*2*) and the decrease in Ku binding to nuclear transporter importins (*3*). Oxidative stress induces the synthesis of Ku70 and Ku80 (*1*). Caspase-3 inhibitor prevents the oxidative stress-induced nuclear loss of Ku proteins and apoptotic cell death. Degraded Ku proteins are expressed via empty circles

a found to bind to the DNA region containing the κB element in the p50 promoter. Therefore, interaction of the Ku antigen with RBP-Jκ and NF-κB p50 may act as a positive regulator for p50 expression in gastric cancer AGS cells. In relation to COX-2, COX-2 expression is mediated by constitutive NF-κB and regulates cell growth and proliferation in human gastric cancer AGS cells. Inactivating Ku70 or Ku80 suppresses cell growth and induces apoptosis. Since both Ku70 and Ku80 expressions are mediated by constitutively activated NF-κB and constitutively expressed COX-2 in gastric cancer cells, both Ku70 and Ku80 expressions may be related to gastric cell proliferation and carcinogenesis. For oxidative DNA damage linked to apoptosis in pancreatitis, oxidative stress may induce a decrease in the Ku70 and Ku80 levels and apoptosis in pancreatic acinar cells. Oxidative stress-induced apoptosis was in parallel with the loss of nuclear Ku proteins in pancreatic acinar cells. Caspase-3 inhibitor prevents the oxidative stress-induced nuclear Ku loss and cell death. Oxidative stress-induced decrease in Ku binding to importin α and importin β reflects a possible modification of the nuclear import of Ku proteins. Therefore, nuclear decrease in Ku70 and Ku80 may result from the decrease in Ku binding to nuclear transporter importins and the degradation of Ku proteins. Nuclear loss of Ku proteins may underlie the mechanism of apoptosis in pancreatic acinar cells after oxidative stress.

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