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The truncation of Ku86 in human lymphocytes

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Abstract

The Ku heterodimer, which consists of Ku70 and Ku86 subunits, is a major sensor of DNA breaks. A truncated form of Ku86 lacking its C-terminus, termed Ku86 variant, has been detected in extracts from different human cells. Here we report that in human lymphocytes the Ku86 variant is not present in vivo but is generated in vitro upon cell lysis by a trypsin-like protease. The resulting Ku86 variant exists exclusively in complexes with Ku70, which possess strong affinity to DNA double strand termini. In different blood donors the levels of Ku86 variant correlated with the magnitude of radiation induced DNA breaks.

Keywords: Ku86 variant; Human lymphocytes; Trypsin-like protease; Radiosensitivity

1. Introduction

Cells induce several metabolic and regulatory pathways in a response to genotoxic insults. Among these pathways are modulation of transcription, activation of DNA repair and cell cycle checkpoints, or induction of cell death by apoptosis. Signal transduction pathways involved in the cellular response to DNA lesions depend on a special class of molecular sensors that detect damaged DNA [1]. Double-strand breaks (DSB) are the most toxic DNA lesions and broken DNA ends are proposed to be a universal signal that triggers the cellular response to genotoxic stress. The major cellular sensor of DSB is Ku protein, a component of the DNA-dependent protein kinase (DNA-PK) [2]. Ku protein, originally identified as an antigen recognized by sera from patients with autoimmune disease [3], is a heterodimer consisting of about 86 kDa (Ku80 or Ku86, hereafter termed Ku86) and 70 kDa (Ku70) subunits [2]. Ku protein binds to DNA termini without sequence specificity [4], and has been shown to possess DNA-dependent ATPase and helicase activities [5,6]. On the other hand, Ku has been reported to be capable of sequence-specific binding within gene regulatory elements, especially in a complex with other transcription factors [7]. Ku protein is indispensable both to the non-homologous end-joining (NHEJ) pathway of DSB repair and to V(D)J recombination in lymphocytes. Cells with mutated Ku proteins exhibit increased sensitivity to ionizing radiation, decreased efficiency of DSB repair, impaired V(D)J recombination and neurogenesis [8]. Both NHEJ and V(D)J recombination require the holo DNA-PK complex, which consists of Ku heterodimer and a 465 kDa serine–threonine protein kinase DNA-PKcs, which is a member of ATM family. Ku protein is a DNA end-binding component of DNA-PK that

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Abbreviations: ATM, ataxia-telangiectasia mutated; DEB, DNA end-binding complex; DNA-PK, DNA-dependent protein kinase; DSB, double strand break; EMSA, electrophoretic mobility-shift assay; MM, multiple myeloma; NHEJ, non-homologous end-joining.

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stimulates the catalytic activity of the kinase [9]. Both Ku70 and Ku86 are necessary for DNA binding [10]. In addition, Ku protein, but not DNA-PKcs is involved in telomere maintenance [11]. Some cellular functions of Ku protein are independent of its DNA-binding activity. Although Ku is a nuclear protein, it can also be detected in cytoplasm. In human B cells a fraction of cytoplasmic Ku interacts with the CD40 membrane receptor and translocates into the nucleus upon IL-4 stimulation [12]. Ku70 also binds to cytoplasmic BAX protein and suppresses its apoptotic translocation to mitochondria [13]. In conclusion, Ku protein is both an important nuclear and cytoprotective factor involved in recombination, repair, transcription and signaling pathways.

It has been reported that in several human cell lines Ku86 protein exists in two forms: full-length ~86 kDa and truncated ~69 kDa. This shorter form of Ku86, termed Ku86 variant, lacks the C-terminus [14,15], which is required for DNA-PKcs activation [16]. Expression of Ku86 variant results in a deficiency of DNA-PK activity due to its impaired ability to recruit the catalytic subunit, even though Ku heterodimer consisting of Ku86 variant still can bind DNA termini [14,15]. The physiological significance of Ku86 variant is not clear at the moment. The presence of Ku86 variant has been reported in extracts from different cell lines, including: B cells from peripheral blood [15], promyeloid leukemia cell line HL60 [14], multiple myeloma (MM) cells [17] and senescent fibroblasts [18]. It has been suggested that in HL60 and MM cells expression of Ku86 variant correlates with increased radio-sensitivity and decreased DNA repair [14,17]. Presently, the mechanisms that contribute to the expression of the Ku86 variant are not clear. Cells containing Ku86 variant do not express additional shorter mRNAs [15], suggesting that a post-transcriptional mechanism may be responsible for Ku86 variant formation. An important mechanism of post-translational protein modification is site-specific proteolysis. It has been reported that truncation of Ku86 in stored cellular extracts could be catalyzed by specific protease that is inhibited by leupeptin [18] or soybean trypsin inhibitor [19], which are specific inhibitors of trypsin-like serine proteases. In the present work we confirm and extend these observations by demonstrating that the variant form of Ku86 does not exist in vivo, but that it is generated upon cell lysis by a specific trypsin-like protease.

2. Materials and methods

2.1. Cells and extracts

Peripheral blood was collected from healthy volunteers 22–44 years old. Lymphocytes were isolated under sterile conditions by centrifugation on a Ficoll gradient (Lymphoprep™, ICN). In some experiments cells were incubated for 24 h in RPMI medium supplemented with 10% of FCS at 37 °C and γ-irradiated on ice in a ^{60}Co beam at a dose of 2 Gy. To stimulate T-cells to proliferate, isolated lymphocytes were cultured for 72 h in the same medium supplemented with lectin (Sigma L-9132; 1 μg/1 ml medium). Leukemia cells (Raji, Jurkats, K562 and HL60) and adenocarcinoma MCF-7 cells were cultured at 37 °C, 5% CO_2 in RPMI medium supplemented with 10% of FCS. PBS-washed cells were suspended in 10 volumes of the lysis buffer consisting of 10 mM Tris (pH 7.6), 1.5 mM $MgCl_2$, 10 mM KCl, 0.5% Nonidet P-40, 1 mM DTT and a protease inhibitor mixture (Complete™, Boehringer) and incubated 15 min with gentle shaking. In some experiments detergent was omitted, and three cycles of freeze-thawing were applied instead. Lysed cells were then centrifuged for 15 min at 500 × *g* and resulting supernatants are referred to as cytoplasmic extracts. Pelleted nuclei were suspended in 5 volumes of a low-salt buffer consisting of 20 mM HEPES pH 7.9, 25% glycerol, 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM EDTA and protease inhibitor mixture (Complete™, Boehringer), and then equal volume of a high-salt buffer (a low-salt buffer supplemented with 0.8 M NaCl) was added. Suspended nuclei were incubated for 15 min on ice, centrifuged for 15 min at 16,000 × *g* and resulting supernatants are referred to as nuclear extracts.

2.2. DNA probes and electrophoretic mobility shift assay (EMSA)

The gel mobility shift analysis was performed as described in detail elsewhere [20]. Briefly, a synthetic double-stranded 36 bp-long oligonucleotide with blunt ends was ^{32}P-5′-end-labeled and purified by polyacrylamide gel electrophoresis. Radioactive oligonucleotide (25 ng) was incubated with cellular extracts (5 μg protein) for 30 min at 4 °C in the binding buffer consisting of 20 mM Tris–HCl (pH 7.6), 5 mM $MgCl_2$, 0.5 mM EDTA, 1 mM DTT, 5% glycerol and 150 mM NaCl. Protein–DNA complexes were formed in a final volume of 20 μl in the presence of 40-fold excess (1 μg) of a non-radioactive plasmid DNA (competitor). Plasmid DNA (pUC19) was either intact (referred to as supercoiled), briefly incubated with DNase I (referred to as nicked) or incubated with Hind III restriction enzyme (referred to as linear). Nucleoprotein complexes were separated by electrophoresis on native 6% polyacrylamide gels in Tris/Borate/EDTA (0.5 ×) buffer. Gels were dried and autoradiographed. Nucleoprotein complexes were quantitated by counting the radioactivity of gel fragments.

2.3. Antibodies and Western blot analyses

Antibodies used were polyclonal goat anti-Ku70 (SC-1486) reactive with the C-terminus of human Ku70 (aa 590–608), polyclonal goat anti Ku86 (SC-1484) reactive with the C-terminus of human Ku86 (aa 710–729) from Santa Cruz Biotechnology (Santa Cruz, CA, USA), and mouse monoclonal anti-Ku86 (clone S10B1) reactive with the N-terminus of human Ku86 (aa 8-221) from Labvision (Fremont, CA, USA). Secondary HRP-conjugated antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Cytoplasmic and nuclear extracts, or total cell lysates (50 μg protein per lane) were separated on 12% polyacrylamide/SDS gels and electrophoretically transferred onto nitrocellulose membranes (Amersham Biosciences or Schleicher and Schuell). Membrane-immobilized proteins were probed with anti-Ku antibodies. The antigen-antibody complexes were visualized using enhanced chemiluminescence Western-blotting detection reagents (Amersham Biosciences).

2.4. Native pore-exclusion limit electrophoresis

Native pore-exclusion limit electrophoresis was performed as described in detail elsewhere [21]. Briefly, nucleoprotein complexes were separated on linear 4–24% polyacrylamide gels in 0.5 × TBE for 16 h at 300 V, ~10 mA at 4 °C. Part of the gel was dried and autoradiographed, the other part was soaked in SDS-Tris–glycine buffer, and then denatured proteins were electrotransferred onto nitrocellulose membranes and probed with antibodies as described above.

2.5. Single cell gel electrophoresis (Comet assay)

Radiation-induced DNA strand-breaks were assessed by alkaline single cell electrophoresis (Comet assay) as described in detail elsewhere [22]. Briefly, lymphocytes suspended in 1% low melting agarose were placed onto microscope slides, lysed for 60 min in cold lysing solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris–HCl (pH 7.5), 1% Triton X-100), denaturated for 20 min in 300 mM NaOH, 1 mM EDTA pH 13 and electrophoresed in the same buffer for 20 min at 1 V/cm. Preparations were neutralized in 0.4 M Tris–HCl (pH 7.5) buffer, stained with ethidium bromide and scored using a fluorescence microscope according to the classification of Collins et al. [23].

3. Results

We have used an electrophoretic mobility-shift assay (EMSA) to detect Ku proteins that bind to free termini of double-stranded oligonucleotide, which mimic DSB in DNA. Two complexes, termed DNA end-binding (DEB)-1 and -2, were detected by EMSA when radio-labeled 36 bp duplex oligonucleotide (designed not to contain binding sites for known transcription factors) was incubated with nuclear extracts from human lymphocytes and promyeloid leukemia cell line K562 (Fig. 1). DEB-1 is by far the major complex formed with K562 extracts, while DEB-2 is the most prominent complex formed in lymphocytes extracts. We show below that these complexes are composed of Ku proteins. DEB-1 and -2 were competed away by linear (L) but not by intact supercoiled (SC) or nicked (OC) non-radioactive DNA species (Fig. 1). Formation of either complex was inhibited in the presence of DNA molecules possessing double-strand ends, indicating that proteins present in the complexes are specific for

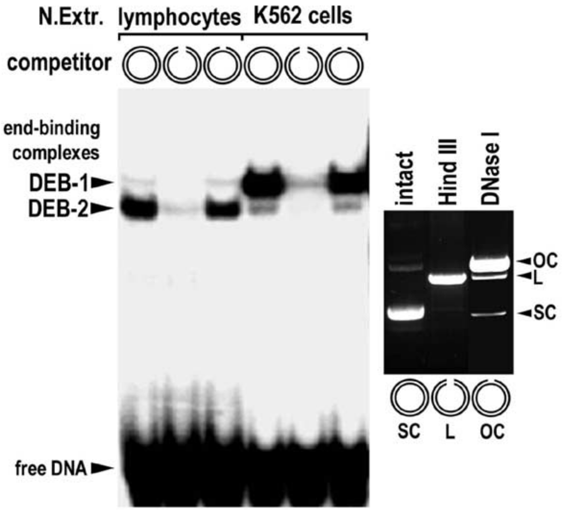

Fig. 1. Detection of DNA end-binding complexes (DEB) in nuclear extracts from human lymphocytes and myeloblastoid K562 cells. Nuclear proteins were incubated with ^{32}P-labeled double-strand oligonucleotide in the presence of excess supercoiled (SC), linear (L) or nicked (OC) plasmid DNA as a competitor, and then nucleoprotein complexes were resolved by gel electrophoresis. Arrowheads represent the positions of DEB complexes and free DNA. The right panel shows the plasmid DNA forms used as a competitor: intact (SC), linearized with Hind III restriction enzyme (L) and nicked with DNaseI (OC).

DSBs. The specificity of such complexes for double-strand ends was further confirmed in competition experiments using a series of plasmid DNA species cleaved to different chain lengths whereby the molarity of DNA ends was varied (data not shown).

To determine the molecular weights and Ku protein compositions of DEB complexes we have employed EMSA using native pore-exclusion limit electrophoresis in combination with Western analyses. Parallel segments of the gel were analyzed for the presence of bound radioactive DNA (DEB activity) and probed with antibodies specific for Ku protein subunits (Fig. 2). We performed the analysis using antibodies against the C-terminus of Ku70, antibodies against the N-terminus of Ku86 (Ku86-NT) and antibodies against the C-terminus of Ku86 (Ku86-CT). Ku70 was present in both DEB complexes. Similarly, antibody against the N-terminus of Ku86 efficiently reacted with both DEB-1 and DEB-2. In contrast, antibody against the C-terminus of Ku86 reacted with DEB-1 but not DEB-2. These results indicate that DEB-1 contains intact Ku86, while DEB-2 contains Ku86 truncated at its C-terminus. DEB-1 and DEB-2 migrated with an estimated mass of about 145 and 160 kDa relative to protein standards, suggesting that Ku heterodimer was the only protein component present in these complexes.

To determine the variation in levels of DEB-1 and DEB-2 complexes among different human cell lines, we performed additional EMSA assays using both cytoplasmic or nuclear extracts. As before, DEB-2 was the most abundant species in lymphocyte nuclear extracts, whereas DEB-1 was most abundant in all the other lines examined with the exception of the cytoplasmic extract from HL60 cells (Fig. 3, upper panel). On the other hand, both DEB-1 and DEB-2 were present in cytoplasmic extracts from such lymphocytes (Fig. 3, upper panel). DEB complexes were barely detectable in extracts from human fibroblasts and HeLa cervical carcinoma cells (data not shown). Western analyses of the same extracts proteins after of denaturing SDS gels was performed using antibodies specific for Ku70 and antibody against the N-terminus of Ku86, which allowed detection of both intact and truncated forms of Ku86

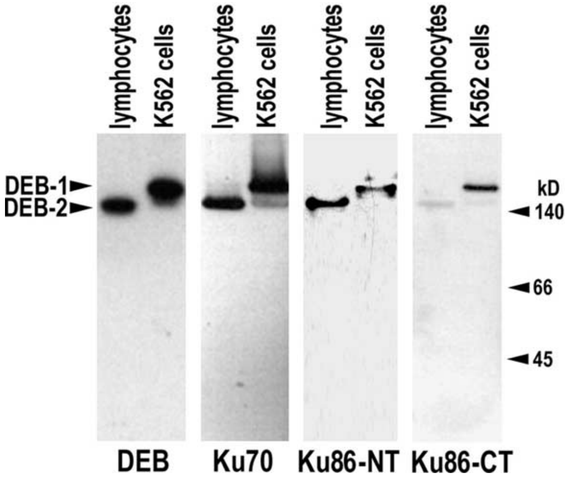

Fig. 2. DNA-end binding complexes contain Ku heterodimers consisting of intact or truncated Ku86. Nucleoprotein complexes were separated using native pore-exclusion limit electrophoresis, and tested for the presence of labeled DNA (DEB) or Western analyzed with antibodies specific for the C-terminus of Ku70 (Ku70), the N-terminus of Ku86 (Ku86-NT) and the C-terminus of Ku86 (Ku86-CT). Arrowheads represent the positions of DEB complexes and molecular mass standards.

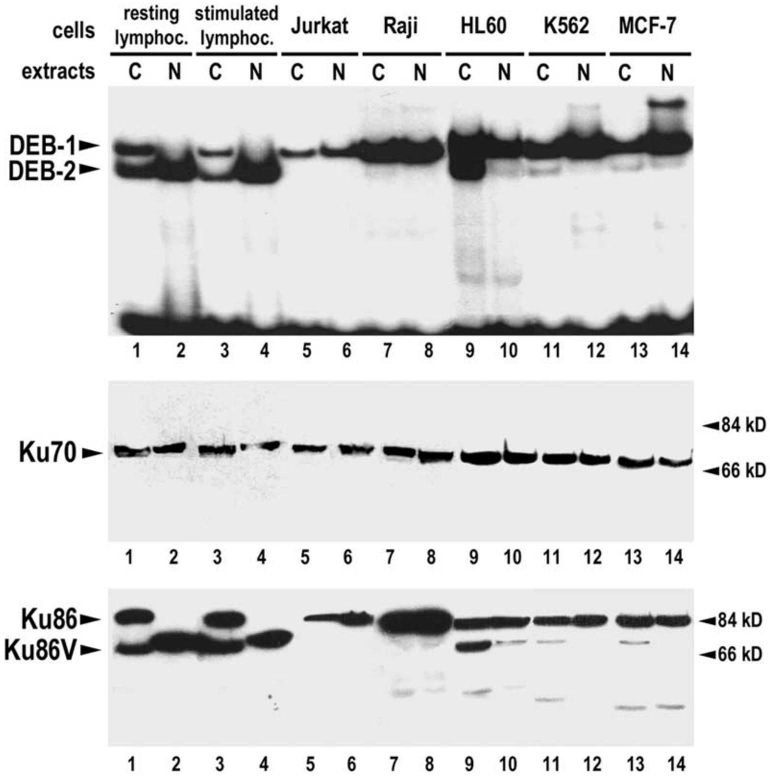

Fig. 3. The truncated variant of Ku86 predominates in extracts from human lymphocytes. Cytoplasmic (C) and nuclear (N) extracts from different human cell lines were tested for DNA-end binding activity (upper panel) and the presence of Ku70 (middle panel) and Ku86 (bottom panel). The truncated variant of Ku86 is depicted as Ku86V. Arrowheads represent the positions of DEB complexes, Ku subunits and molecular mass standards.

(Fig. 3). The full-length Ku70 was present in all tested extracts possessing DEB activity (Fig. 3, middle panel). As expected, two forms of Ku86 were present in these extracts; the shorter form of Ku86 which is truncated at its C-terminus migrated with an average mass of 69 kDa and apparently was identical with Ku86 variant described elsewhere in the literature [14,15], hereafter termed Ku86V. Ku86V has been detected in all extracts possessing DEB-2 activity (Fig. 3, bottom panel), in line with presumption that DEB-2 consist of Ku70/Ku86V heterodimer.

We further investigated the variation in DEB-1/DEB-2 levels (and Ku86/Ku86V) in lymphocytes from the blood of several donors. When nuclear extracts from resting lymphocytes were tested, the vast majority of DEB activity corresponded to DEB-2 (90% on the average, ranging from 70 to 100% in eight different samples)(data not shown). In contrast, both DEB-1 and DEB-2 contributed more similarly to total DEB activity when cytoplasmic extracts were examined)(data not shown). In such extracts DEB-2 represented about 55% of total DEB activity, on the average (ranged from 25 to 90%). For comparison, DEB-2 represent about 15 and 5% of total DEB activity in nuclear and cytoplasmic extracts from K562 cells)(data not shown). We have also tested possible changes in the DEB activity in lymphocytes gamma-irradiated at the dose of 2 Gy compared to corresponding not irradiated cells. However, we have not observed any significant changes in either the relative proportion of DEB-1/DEB-2, their levels or distribution among sub-cellular fractions, neither directly after irradiation nor 4 h later (data not shown).

One can postulate that truncation of Ku86 takes place in living cells. Alternatively, this process might happen during the course of preparation of cellular extracts suitable for EMSA experiments (in spite of the wide-range protease inhibitors included to all solutions). To clarify these issues, we have assayed the levels of Ku86V in cellular extracts and in total cell preparations obtained by direct lysis and denaturation of cells in SDS-buffer. We have performed such analyses on mononuclear cells directly after their purification from peripheral blood (about 80% T-cells, 20% B-cells and traces of monocytes), peripheral blood lymphocytes after their 24 h incubation in non-stimulating medium, and for proliferating T-cells after 3 days incubation in stimulating medium containing lectin. Fig. 4A shows that only traces of Ku86V are present in whole cell preparations, irrespective of the cell source. This result indicates that proteolysis of Ku86 takes place during lysis and sub-cellular fractionation of lymphocytes. The proportions of Ku86/Ku86V were similar in extracts from lymphocytes directly isolated from the blood and lymphocytes cultured in non-stimulating medium. Cytoplasmic extracts from T-cells cultured for 3 days in proliferation-stimulating medium contained slightly higher amounts of the intact Ku86 (5–20% more as compared to resting lymphocytes, depending on the blood donor; data not shown). Irradiation of lymphocytes with a 2 Gy dose did not induce Ku86 truncation in vivo when whole cell preparations were studied as described above (within the time period from 0 to 120 after irradiation, data not shown).

In our experiments cells have been lysed in the presence of non-ionic detergent (NP40), leaving open the possibility that the putative protease(s) responsible for Ku86 truncation could be either membrane associated or enclosed. To test this possibility, lymphocytes were lysed by repeated freeze-thaw cycles in the absence of detergent. However, such non-detergent lysis did not prevent truncation of Ku86 (Fig. 4B, lanes 3 and 4). All buffers used for cell lysis and fractionation were supplemented with a mixture of wide-range-acting protease inhibitors at concentrations sufficient to prevent general proteolysis. Proteolytic cleavage of Ku86 in such conditions suggests that the putative protease(s) specific for Ku86 is relatively resistant to typical inhibitors. It had been shown that proteolysis of Ku86 was inhibited by 5 μM leupeptin when extracts from senescent human fibroblasts were tested [18]. However, when lymphocytes were isolated and lysed in the presence of 10 μM leupeptin the activity of the putative Ku86-specific protease(s) was unchanged (Fig. 4B, lanes 5and6). More recently it had been reported that a degradation of Ku86 mediated by nuclear extracts from conditioned lymphocytes was reduced in the presence of the soybean trypsin inhibitor [19]. Here we have studied the effects of this inhibitor on Ku86 integrity when added to isolation and lysis buffers.

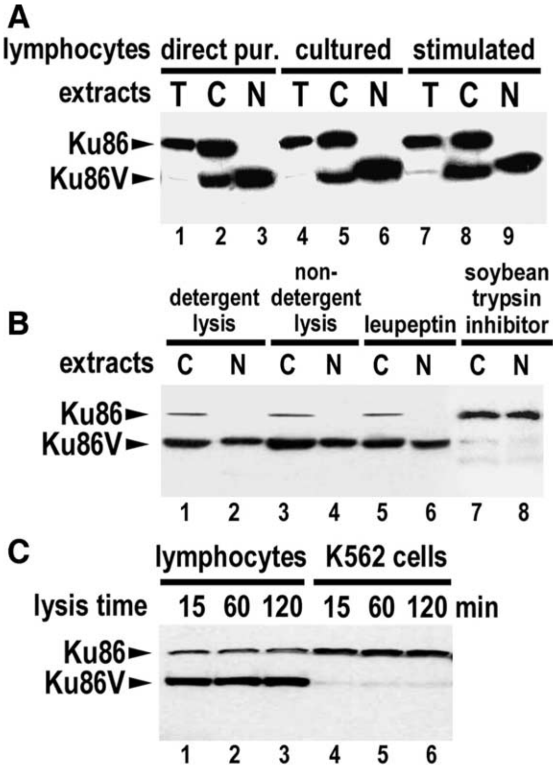

Fig. 4. Truncation of Ku86 depends on a trypsin-like serine protease activated during lysis and fractionation of human lymphocytes. Panel A: the presence of intact and truncated variants of Ku86 in total cell lysates (T) and cytoplasmic (C) or nuclear (N) extracts from freshly isolated human mononuclear blood cells (direct pur.), resting lymphocytes incubated for 24 h in non-stimulating medium (cultured) and proliferating T lymphocytes incubated for 3 days in medium containing lectin (stimulated). Panel B: the presence of intact and truncated variants of Ku86 in cytoplasmic (C) and nuclear (N) extracts from resting human lymphocytes lysed by NP40 treatment (detergent lysis) or repeated freeze-thawing (non-detergent lysis), and lysed by NP40 in the presence of leupeptin (10 μM) or soybean trypsin inhibitor (100 μM). Panel C: the presence of intact and truncated variants of Ku86 in cytoplasmic extracts from human lymphocytes and myeloblastoid K562 cells after prolonged incubation of cell lysates.

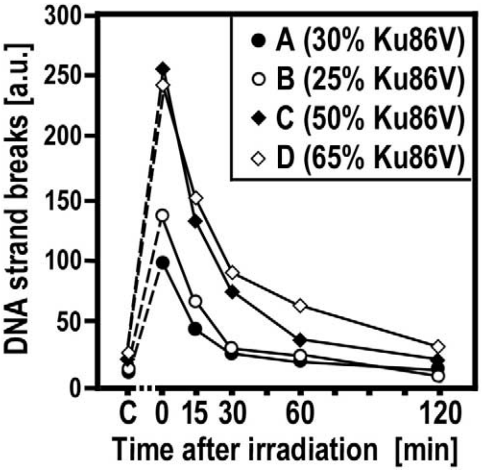

Fig. 5. The levels of radiation-induced DNA strand breaks determined by the Comet Assay in lymphocytes of donors with different capacities to generate Ku86V in vitro. DNA breaks were analyzed in either control non-irradiated cells (C), directly after irradiation (0) or at different time periods (15, 30, 60 and 120 min) after irradiation. Numbers shown in the legend represents relative amounts of Ku86V in cytoplasmic extracts from four donors A, B, C and D.

Supplementation of such buffers with this protein (at 100 μM concentration) fully prevented proteolysis of Ku86 (Fig. 4B, lanes 7 and 8), indicating that the putative Ku86-specific protease(s) belongs to the family of trypsin-like serine proteases.

We have also studied the kinetics of Ku86 proteolysis in cellular extracts. To do so, lymphocytes and K562 cells were incubated on ice in lysis buffer for different time periods (from 15 min to 2 h), and then Ku86 forms in cytoplasmic extracts were assayed for in Western blots. Fig. 4C shows that neither the proportion of Ku86/Ku86V in lymphocytes has been changed nor Ku86V has been generated in K562 cells during such incubation. This data indicates that proteolysis of Ku86 in lysed lymphocytes goes fast to completion but a detectable fraction of Ku86 remains unmodified.

Reports by others [14,17] together with our results suggest that the capacity of cells to generate Ku86V in vitro may correlate with increased radio-sensitivity and decreased DNA repair in vivo. To verify this hypothesis, lymphocytes from four donors whose levels of Ku86V in cytoplasmic extracts differed significantly have been tested for the levels of radiation-induced DNA breaks and capacity for DNA repair using the alkaline single-cell electrophoresis test (Comet test). Data presented in Fig. 5 show that the levels of initial DNA breaks and breaks detected after cell recovery are significantly lower in lymphocytes isolated from two donors with lower capacities to generate Ku86V in vitro (donors A and B, about 30 and 25% of DEB-2, respectively) as compared to donors with higher amounts of Ku86V (donors C and D, about 50 and 65 of DEB-2, respectively). We conclude that DNA repair is compromised in cells that possess the capacity to generate more Ku86V in vitro.

4. Discussion

The 69 kDa truncated form of Ku86, which lacks the C-terminus, termed Ku86 variant, predominates in protein extracts from human lymphocytes. Although such Ku86 variant is not present in living cells, a trypsin-like protease is activated upon the lymphocyte lysis which catalyzes cleavage of Ku86 (Fig. 4). Based on the aminoacid sequence and the size of truncated Ku86 we propose that putative cleavage site localizes within the stretch of basic residues (Arg-599, Lys-603, Lys-605, Lys-606), upstream of the Ku86 nuclear localization signal (residues 561–569, [24]). Both intact and truncated forms of Ku86 form complexes with Ku70 that efficiently bind DNA termini, which is evidenced by EMSA experiments (Figs. 1–3). The ability of Ku86V-containing Ku heterodimers to bind DNA termini contrasts with their impaired ability to stimulate DNA-PK activity reported elsewhere [14,15], which is consistent with the role of C-termini of Ku86 in the recruitment of DNA-PK catalytic subunit [16]. Ku86 proteolysis in the cytoplasmic fraction is limited because the intact form is also present (Fig. 4C). This indicates that either some fraction of Ku86 is resistant to the protease(s), or that the protease(s) is rapidly inactivated by autolysis, or both. Although these mechanisms are not clear at present, cell compartmentalization, post-translational modification (e.g. site-specific phosphorylation or acetylation), or formation of complexes with other proteins may contribute to putative resistance of Ku86.

The proteolytic processing of Ku86 to the truncated form is nearly quantitative in the nuclear fraction. One can postulate three alternative explanations for that phenomenon: (i) the activity of the protease(s) is higher in the nuclear compartment; (ii) a protease-resistant fraction of Ku86 is higher in a cytoplasmic compartment; (iii) Ku70/Ku86V heterodimer firmly binds chromatin and is more resistant to leakage from nuclei upon cell fractionation as compared to Ku70/Ku86 heterodimer. It is now appreciated that several nuclear proteins are easily lost from nuclei upon cell lysis and subcellular fractionation [21]. One could postulate, that a large fraction of Ku detected in cytoplasmic extracts represents nuclear Ku protein loosely attached to the chromatin. For this reason any estimation of a cytoplasmic/nuclear distribution of the protease-resistant Ku86 form that based on Ku86V content in subcellular fractions might be misleading.

Physiological factors affecting the truncation of Ku86 have not been determined. It has been reported that proteolysis of Ku86 in extracts from conditioned lymphocytes did not correlate with decreased cell viability or increased apoptosis of the cells [19]. On the other hand, Ku86 in extracts from proliferating T-cells showed decreased proteolysis [19], suggesting that growth processes may affect protease activity. It had been postulated by others that high amounts of Ku86 variant detected in extracts from myeloblastoid cells was correlated with increased sensitivity to ionizing radiation [14,17]. In agreement with this hypothesis, we have observed that low amounts of Ku86V in cellular extracts correlated with low levels of DNA breaks induced by ionizing radiation in lymphocytes. We have no evidence for increased radiation-induced truncation of Ku86. However, some proteolysis of Ku86 possibly happens in living lymphocytes and the presence of the truncated form of Ku86 might contribute to cellular radiosensitivity. One could speculate, that Ku70/Ku86V heterodimers bind with high affinity to DNA breaks and compete with intact Ku70/Ku86 heterodimers' ability to recruit DNA-PKcs to damaged sites. The hypothetical mechanism would be similar to the competition between intact PARP-1 and a caspase-generated DNA-binding fragment of PARP-1 that lacks catalytic activity [25].

Acknowledgements

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References

[1] B.-B.S. Zhou, S.J. Elledge, The DNA damage response: putting checkpoints in perspective, Nature 408 (2000) 433–439.

[2] G.C.M. Smith, S.P. Jackson, The DNA-dependent protein kinase, Genes Dev. 13 (1999) 916–934.

[3] T. Mimori, M. Akizuki, H. Yamagata, S. Inada, S. Yoshida, M. Homma, Characterization of a high molecular weight acidic nuclear protein recognized by autoantibodies in sera from patients with polymyositis scleroderma overlap, J. Clin. Invest. 68 (1981) 611–620.

[4] X. Wu, M.R. Lieber, Protein–protein and protein–DNA interaction regions within the DNA end-binding protein Ku70–Ku86, Mol. Cell. Biol. 16 (1996) 5186–5193.

[5] Q.P. Cao, S. Pitt, J. Leszyk, E.F. Baril, DNA-dependent ATPase from HeLa cells is related to human Ku autoantigen, Biochemistry 33 (1994) 8548–8557.

[6] N. Tuteja, R. Tuteja, A. Ochem, P. Taneja, N.W. Huang, A. Simoncsits, et al., Human DNA helicase II a novel DNA unwinding enzyme identified as the Ku autoantigen, Eur. Mol. Biol. Org. J. 13 (1994) 4991–5001.

[7] D.M. Willis, A.P. Loewy, N. Charlton-Kachigian, J.S. Shao, D.M. Ornitz, D.A. Towler, Regulation of osteocalcin gene expression by a novel Ku antigen transcription factor complex, J. Biol. Chem. 277 (2002) 37280–37291.

[8] Y. Gu, J. Sekiguchi, Y. Gao, P. Dikkes, K. Frank, D. Ferguson, et al., Defective embryonic neurogenesis in Ku-deficient but not DNA-dependent protein kinase catalytic subunit-deficient mice, Proc. Natl Acad. Sci. USA 97 (2000) 2668–2673.

[9] T.M. Gottlieb, S.P. Jackson, The DNA-dependent protein kinase: requirement for DNA ends and association with Ku antigen, Cell 72 (1993) 131–142.

[10] J. Wang, X. Dong, W.H. Reeves, A model for Ku heterodimer assembly and interaction with DNA, J. Biol. Chem. 273 (1998) 31068–31074.

[11] H.-L. Hsu, D. Gilley, S.A. Galande, M.P. Hande, B. Allen, S.-H. Kim, et al., Ku acts in a unique way at the mammalian telomere to prevent end joining, Genes Dev. 14 (2000) 2807–2812.

[12] T. Mirio, S.H. Hanissian, L.B. Bacharier, H. Teraoka, S. Nonoyama, M. Seki, et al., Ku in the cytoplasm associates with CD40 in human B cells and translocates into the nucleus following incubation with IL-4 and anti-CD40 mAb, Immunity 11 (1999) 339–348.

[13] M. Sawada, P. Hayes, S. Matsuyama, Cytoprotective membrane-permeable peptides designed from the Bax-binding domain of Ku70, Nat. Cell Biol. 5 (2003) 352–357.

[14] Z. Han, C. Johnston, W.H. Reeves, T. Carter, J.H. Wyche, E.A. Hendrickson, Characterization of a Ku86 variant protein that results in altered DNA binding and diminished DNA-dependent protein kinase activity, J. Biol. Chem. 271 (1996) 14098–14104.

[15] C. Muller, C. Dusseau, P. Calsou, B. Salles, Human normal peripheral blood B-lymphocytes are deficient in DNA-dependent protein kinase activity due to the expression of a variant form of the Ku86 protein, Oncogene 16 (1998) 1553–1560.

[16] B.K. Singleton, M.I. Torres-Arzayus, S.T. Rottinghaus, G.E. Taccioli, P.A. Jeggo, The C terminus of Ku80 activates the DNA-dependent protein kinase catalytic subunit, Mol. Cell. Biol. 19 (1999) 3267–3277.

[17] Y.T. Tai, G. Teoh, B. Lin, F.E. Davies, D. Chauchan, S.P. Treon, et al., Ku86 variant expression and function in multiple myeloma cells is associated with increased sensitivity to DNA damage, J. Immunol. 165 (2000) 6347–6355.

[18] Y.W. Jeng, H.C. Chao, C.F. Chiu, W.G. Chou, Senescent human fibroblasts have elevated Ku86 proteolytic cleavage activity, Mutat. Res. 435 (1999) 225–232.

[19] A. Sallmyr, L. Du, A. Bredberg, An inducible Ku86-degrading serine protease in human cells, Biochim. Biophys. Acta 1593 (2002) 57–68.

[20] J. Łanuszewska, A. Cudak, J. Rzeszowska-Wolny, P. Widlak, Detection of damage-recognition proteins from human lymphocytes, Acta Biochim. Pol. 47 (2000) 443–450.

[21] P. Widlak, J. Łanuszewska, R.B. Cary, W.T. Garrard, Subunit structures and stoichiometries of human DFF proteins before and after induction of apoptosis, J. Biol. Chem. 278 (2003) 26915–26922.

[22] M. Green, J. Low, S.A. Harcourt, P. Akinluyi, T. Row, J. Cole, et al., UV-C sensitivity of unstimulated and stimulated human lymphocytes from normal and *Xeroderma pigmentosum* donors in the comet assay: a potential diagnostic technique, Mutat. Res. 273 (1992) 137–144.

[23] A. Collins, I. Fleming, C. Gedik, In vitro repair of oxidative and ultraviolet-induced DNA damage in supercoiled nucleoid DNA by human cell extract, Biochem. Biophys. Acta 1219 (1994) 724–727.

[24] M. Koike, T. Ikuta, T. Miyasaka, T. Shiomi, Ku80 can translocate to the nucleus independent of the translocation of Ku70 using its own nuclear localization signal, Oncogene 18 (1999) 7495–7505.

[25] D. D'Amours, F.R. Sallmann, V.M. Dixit, G.G. Poirier, Gain-of-function of poly(ADP-ribose) polymerase-1 upon cleavage by apoptotic proteases: implications for apoptosis, J. Cell. Sci. 114 (2001) 3771–3778.