Virchows Arch (2001) 438:146–153
DOI 10.1007/s004280000334

ORIGINAL ARTICLE

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Radiation sclerosing proliferative atypical nephropathy of peritumoral tissue of renal-cell carcinomas after the Chernobyl accident in Ukraine

Received: 11 January 2000 / Accepted: 22 September 2000 / Published online: 7 December 2000

Abstract After the Chernobyl accident, the morbidity of renal-cell carcinomas in Ukraine increased gradually from 4.7 to 7.5 per 100,000 of the total population. Cesium 137 (^{137}Cs) is responsible for 80–90% of the internal radioactivity in people living in radiocontaminated areas of Ukraine, and 90% of ^{137}Cs is eliminated through the kidneys. Histological and immunohistochemical study of proliferating cell nuclear antigen (PCNA) and K-*ras* protein was performed in peritumoral kidney tissues of 167 Ukrainian patients (groups I–III, according to varying degrees of internal exposure to radiation), and of 85 analog Spanish patients, as a control group. Our data showed in the majority of Ukrainian patients a radiation sclerosing proliferative atypical nephropathy (RSPAN) in association with an increase in the incidences of tubular epithelial nuclear atypia and carcinoma in situ (CIS). Areas of epithelial nuclear atypia and CIS of the cortex and medulla showed significant PCNA expression with means of extent as 12, 14, and 15% of stained nuclei in groups I, II, and III respectively. K-*ras* expression of the same areas occurred in 67, 87, and 85% of cases in groups I, II, and III respectively. The present study points to a strong relationship between the long term of low-dose radiation exposure of the Ukrainian population and the development of RSPAN as a possible precursor of malignancy. In addition, it confirms the possible initiator, promoter, or progressor role of chronic low-level radiation of renal human carcinogenesis in Ukraine.

Keywords Renal-cell human carcinogenesis · Chronic low-dose radiation · Chronic radiation nephropathy

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Introduction

In the early days of radiation research, the kidney was known to have a low relative sensitivity to radiation induction of cancer [23, 42]. Recent literature reviews documented the significant risk of experimental radiation-induced renal cancer [7, 13, 50]. Furthermore, there are more than four cases describing the clinical features of radiation-induced renal-cell carcinomas (RCCs) in humans following earlier therapeutic radiation therapy [44].

Currently, public concern over radiation safety has become even more acute with the growth of the nuclear power industry and recent accidents at nuclear power plants, such as Chernobyl (April, 1986). As of now, 14 years after the Chernobyl accident (70 km from Kiev City), more than ten million people, who live in the radiocontaminated areas, have been chronically exposed to low doses of still-existing ionizing radiation. It is necessary to note that 90% of Cesium 137 (^{137}Cs), which is responsible for most of the exposure in the Ukrainian population, is concentrated and eliminated through the kidneys [27]. Morbidity of malignant renal tumors in adults during the period 1986–1998 has increased from 4.7 to 7.5 per 100,000 of the total population (from 6.0 to 9.8 per 100,000 of the male population and from 3.6 to 5.5 per 100,000 of the female population) in Ukraine [45].

The term “low doses” (0.1–0.5 Gy of radiation) means to be above background levels yet below that which could induce acute effects usually associated with cell death [37]. Recently, it has been suggested that low-dose ionizing radiation has the potential to act as a mitogen or tumor promoter [14, 36].

Proliferating cell nuclear antigen (PCNA) plays an essential role in nucleic acid metabolism as a component of the replication and repair machinery. The protein has been located in the nucleus of cells that are in the S phase of the cell cycle and in irradiated non-S-phase cells, which suggests the involvement of the protein in DNA repair processes [16]. The normal kidney has a low rate of proliferation, as it reflects in analysis of some

Table 1 Patient characteristics. Contamination data in the area (Ci/km^2) are from Raes et al. [26]. *NC* non-contaminated areas

	Group I	Group II	Group III	Group IV
No. of patients	42	69	56	85
Mean age (years; range)	55 (27–79)	57 (34–75)	55 (29–77)	58 (26–77)
Contamination level	NC	0.5–5	5–30	NC

benign tissue adjacent to the renal carcinomas that reveals most of the tubular epithelium negative against PCNA and scanty cases with less than 1% of PCNA-positive nuclei [28].

Ras genes encode a group of 21-kDa membrane-associated proteins (p21) that are involved in the control of cell proliferation. *Ras* proteins bind guanosine triphosphate (GTP) and guanosine diphosphate (GDP) and have an intrinsic GTPase activity. They may function like regulatory G proteins and control cell proliferation by regulating signal transduction pathways at the plasma membrane [6]. To our knowledge, there are no bibliographic references on immunohistochemical (IHC) *ras* protein expression in normal or peritumoral kidney.

We investigated the histological and the PCNA and K-*ras* IHC changes of peritumoral kidney tissue, which could be associated with radiation exposure in the Ukrainian population. We selected three groups of patients with RCC in Ukraine that represent varying degrees of internal exposure to radiation. The Spanish group of analogous patients with RCC represents the control group for the present study.

Materials and methods

Peritumoral kidney tissue samples

Without previous treatment, 348 patients from Ukraine and Spain underwent radical nephrectomy due to RCC. According to the level of radiocontamination, the 236 patients from Ukraine, who underwent surgery in the Institute of Urology and Nephrology in Kiev, were selected as groups I–III. A Spanish group of 112 patients who underwent surgery in the University Clinic Hospital of Valencia represented control group IV. The results of histological analysis of RCCs of these patients according to the Heidelberg International Union Against Cancer (UICC) classification [3] are presented in another paper [31]. The number of slides retrieved per case ranged from 4 to 11, with an average of seven slides per case. As has been recommended recently [20], we included in most cases peritumoral samples from the interface between the tumor and the normal kidney and a block of normal kidney away from the tumor to include cortex, medulla, and associated papilla. Microscopic analysis of formalin-fixed paraffin-embedded tissue showed severe degenerative, atrophic and/or inflammatory changes in 97 Ukrainian and 27 Spanish peritumoral samples and, thus, these cases were ruled out. Hence, we selected for our study 167 Ukrainian and 85 Spanish cases of peritumoral kidney. Characteristics of all patients are summarized in Table 1.

The histological study was performed with analysis of the cortex, medulla, and interstitium. Glomerular basement membranes, the presence of degenerative (nephrosis-like) changes with necrobiosis, and/or necrosis of tubular epithelium, nuclear tubular pyknosis, nuclear tubular atypia, and/or carcinoma in situ (CIS), were evaluated in the cortex. Analysis of the medullar compartment included collecting ducts with the evaluation of desquamative, regenerative changes, naked wide ducts, and nuclear pyknosis, nuclear atypia, and/or CIS. The presence and intensity of the interstitial inflammatory response and the state of stroma (edematous, hemorrhagic, and sclerotic changes) were also carefully studied. Nuclear atypia was diagnosed when tubular epithelial cells showed diverse alterations, such as loss of nuclear uniformity in shape and in arrangements, changes in chromatin distribution, from nuclear vacuolization to condensation, and a nuclear/cytoplasmatic increased ratio. CIS showed tubular epithelium with one line of cells having large strong hyperchromatic irregular nuclei with frequent prominent nucleoli and scanty cytoplasm.

IHC staining

IHC staining was performed using a standard avidin biotin peroxidase complex (ABC) method with a LSAB kit (Dako, Denmark). Serial sections were deparaffinized, and endogenous peroxidase activity was blocked with 3% hydrogen peroxide in distilled water for 5 min. Sections were heated 3–5 min in an autoclave (1–1.5 atmospheres) in citrate buffer, pH 6.1, for 30 min for antigen retrieval. Nonspecific binding was blocked with 5% normal horse serum in phosphate-buffered saline (PBS) at 37°C for 30 min. Incubation was with monoclonal antibodies to PCNA antimouse antibody (PC-10, IgG2a, Dako) at a 1:50 dilution and K-*ras* antirabbit polyclonal antibody (Santa Cruz Biotechnology, Inc., Calif.) at a 1:100 dilution for 1 h at room temperature. As controls, known positive tissue sections were used and, for negative controls, exposure to the primary antibody was omitted. Color was developed using 3,3'-diaminobenzidine, and counter staining with Mayer's hematoxylin for 1 min was applied. The IHC analysis was performed in a blind fashion without knowledge of the patient group. All specimens were evaluated independently by at least two pathologists.

Quantitative analysis

Histological analysis of peritumoral tissue lesions was performed using a semiquantitative scale for evaluation of variables. Each case was evaluated as 0 when the finding for the study was negative and as 1, 2, or 3 when the same finding occurred in less than 10%, more than 10% but less than 50%, and more than 50%, respectively. IHC analysis was performed using a system for evaluation and grading of the immunostaining patterns with values for intensity, distribution, and extent. The intensity of staining was scored using the following criteria: no detectable staining, low, moderate, and strong staining. The extent of staining was scored as: 0, no detectable staining; low, less than 10% scattered stained cells; moderate, more than 10% but less than 50% stained cells; strong, homogeneous staining in more than 50% of cells. The percentage of cells expressing PCNA was determined by counting 1000 cells per slide.

Statistical analysis

For analysis of variables, each had a qualitative or quantitative connotation, depending on their possibility for evaluation. Ages of patients and PCNA expression in percentage had a quantitative variable, and the other histological and immunohistochemical variables had a qualitative connotation. In order to clarify the findings, we considered them positive when occurring in more than 10% of histological or cellular structures and negative when not present or minimally positive (less than 10% of histological or cellular structures). Statistical analysis was performed in order to

Table 2 Incidences of irradiation nephropathy lesions in peritumoral kidney of Ukrainian groups

Irradiational characteristics	Groups			Statistical analysis
	I	II	III	
Cortex				
Tubular nuclear pyknosis	21 (51%)	38 (56%)	36 (67%)	χ^2=1.88, ns
Degeneration	31 (75%)	59 (86%)	50 (90%)	χ^2=4.48, ns
Thickening of glomerular basement membranes	10 (24%)	52 (76%)	45 (81%)	χ^2=36.96, $P<10^{-5}$
Collecting ducts				
Nuclear pyknosis	33 (78%)	67 (97%)	55 (98%)	χ^2=13.75, $P<0.01$
Regeneration	34 (80%)	64 (92%)	47 (83%)	χ^2=3.8, ns
Desquamation	31 (73%)	57 (82%)	49 (87%)	χ^2=3.08, ns
Naked wide ducts	26 (61%)	50 (72%)	45 (80%)	χ^2=4.09, ns
Interstitium				
Edema	14 (33%)	4 (5%)	10 (17%)	χ^2=14.26, $P<0.001$
Sclerosis	24 (57%)	50 (72%)	37 (66%)	χ^2=2.76, ns
Inflammation	7 (16%)	26 (37%)	17 (30%)	χ^2=5.50, ns

determine whether pathological and immunohistochemical data were related with each other and with the different groups of patients. We applied the χ^2 test for statistical analysis between qualitative variables; Fisher's test and Student's *t*-test were used to assess the relationships between qualitative and quantitative variables.

Results

Histological study of the majority of cases from the Ukrainian groups showed glomeruli frequently enlarged with decreased lobulation and thickening of the capillary walls with obstruction of loops, occasionally containing pyknotic cells, simulating membranous nephropathy. Tubular epithelia of cortex presented multiple nuclear pyknosis, occasionally with eosinophilic cytoplasm, and focally tubular degeneration and disorganization. Connective tissue also contained some pyknotic cells. The quantity and frequency of cortical lesions increased from group I to III, some with significant differences (Table 2).

The majority of cases from the Ukrainian groups demonstrated large areas of pyknotic cells and epithelial detachment, degeneration, regeneration, and desquamation in collecting ducts (Table 2). Pyknotic changes were more frequent in groups II and III than in group I, with significant differences. Many cases showed the collecting ducts filled with desquamated garlands of hyperchromic regenerated epithelium, and the widened, enlarged loops of collecting ducts were already naked and empty, resembling "cheese holes" (Fig. 1). Moreover, multiple foci of cortical and medullar tubular nuclear atypia were observed in the majority of cases in groups II and III and less in I, in comparison with group IV of the Spanish patients. In 19, 29, and 36% of cases, the tubules were occasionally filled with severe atypical cells mimicking CIS in groups I, II, and III, respectively. Only one CIS from eight cases with nuclear atypia of group IV was detected (Table 3).

The medullar interstitium was edematous or more frequently apparent as large areas of sclerosis. Active inflammation was not typically observed. The majority of the Ukrainian cases demonstrated medullar patchy hemorrhage and were hypervascularized with multiple wide microvessels and the development of angiomatoid-like vessels.

Only in the Ukrainian groups were we able to detect changes in glomerular basement membranes, in tubular epithelia of cortex and medulla (degeneration and desquamation, regeneration, and nuclear pyknosis), and in interstitium. As a whole, we termed these changes irradiation-like nephropathy; their quantity and frequency increased in patients who live in radiocontaminated areas (groups II and III).

IHC analysis of 116 kidney peritumoral tissues demonstrated a dramatic increase of nuclear PCNA staining in proximal and distal tubular epithelium, with an average extent of 12, 14, and 15% of PCNA-stained tubular epithelial nuclei in groups I, II, and III, in comparison with minimal staining in group IV, wherein only seven cases revealed positivity, with a mean of staining nuclei of 1.8%. Areas of epithelial nuclear atypia and CIS of the cortex and medulla showed strong intensive nuclear staining, which did not occur in areas of irradiation-like lesions of medulla in the desquamated garlands of regenerative epithelium of collecting ducts (Fig. 2).

A significant increase of cytoplasmic homogenous and especially membranous K-*ras* protein expression of proximal, distal tubules, and collecting ducts in peritumoral kidney occurred in 67, 87, and 85% of cases in groups I, II and III, respectively. The percentage of positive cells was predominantly between 25% and 50%, without significant differences between groups. The moderate

Fig. 1 Peritumoral kidney tissues of Ukrainian patients exposed to long-term, low-dose ionizing radiation. **A**, **B** Cortical lesions with nuclear pyknosis, areas of nuclear atypia, and degeneration of tubular epithelium. **C**, **D** Medullar lesions with regeneration and detachment of collecting duct epithelium, hypervascularization of interstium, pyknosis, and nuclear atypia. **E**, **F** Carcinoma in situ of collecting ducts. Hematoxylin and eosin; magnification **A**, **E** ×230; **C** ×100; **B**, **D**, **F** ×400) ▶

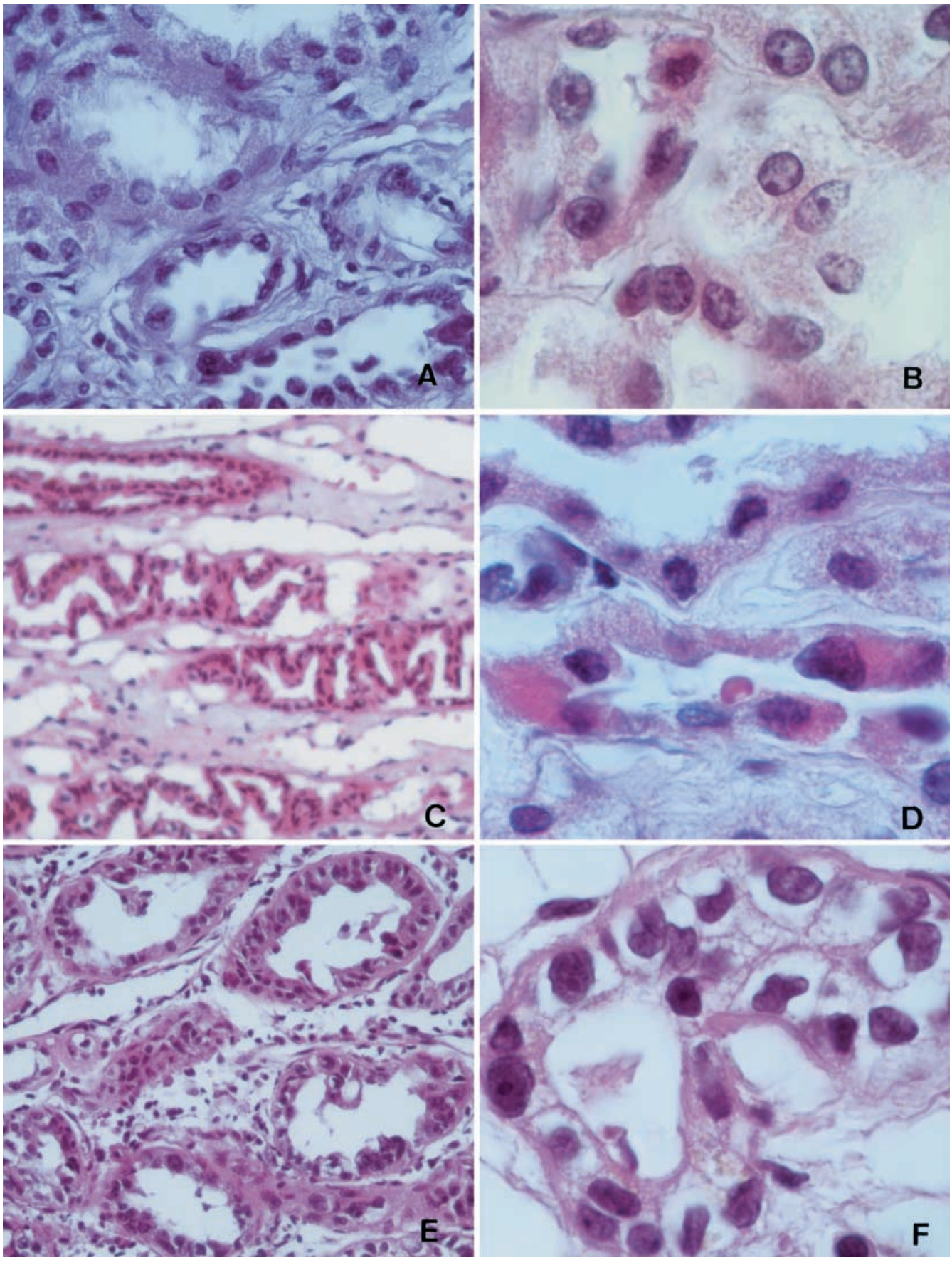
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Table 3 Incidences of peritumoral kidney epithelial nuclear atypia and carcinoma in situ (CIS) in the different groups. χ^2=80.8, P<0.001

Groups	No. of cases	Nuclear atypia (%)	CIS (%)
I	42	17 (40%)	8 (19%)
II	69	52 (75%)	20 (29%)
III	56	40 (71%)	20 (36%)
IV	85	8 (7%)	1 (0.8%)

and strong K-*ras* expression directly correlated with an increase of nuclear pyknosis and regenerative changes of tubular and collecting duct epithelium. Strong, predominantly membranous epithelial K-*ras* staining of widened medullar collecting ducts with irradiation-like lesions was detected in the majority of peritumoral tissues of groups II and III (Fig. 2). Moreover, areas of epithelial nuclear atypia or CIS were more frequently K-*ras*-positive stained (χ^2=20.9; P<0.05).

Discussion

These results suggest for the first time that chronic long-term (more than 14 years) low-dose ionizing radiation in humans could influence renal carcinogenesis. Our data provide compelling evidence for a dramatic increase in the incidences of epithelial nuclear atypia and CIS of the peritumoral cortical, and especially medullar tissue in association with chronic radiation nephropathy in patients who live in the most radiocontaminated areas.

Our recent studies concerning urinary bladder urothelium biopsied from 45 male patients with benign prostate hyperplasia without any bladder symptoms, living in radiocontaminated areas of Ukraine, demonstrated frequent severe urothelial dysplasia, CIS, and a single small invasive transitional cell carcinoma combined with irradiation cystitis in 42 cases (93%) [29, 47].

Intratubular epithelial nuclear atypia has not been fully described in human RCC. Recently, “dysplastic” tubular epithelium in “normal” kidney and CIS associated with RCC have been regarded as precursors of RCC, but their incidence has never exceeded 23% of tumors, the lesions being predominantly cortical and periglomerular [18, 22, 48]. Our results revealed two types of cellular lesions. The first type constitutes radiation-like lesions, which represent changes in cortical tubular epithelium and more frequently in medullar collecting ducts, such as nuclear pyknosis, vacuolization, degeneration, desquamation, regeneration, and proliferation, all of which added to vascular and interstitial lesions constituting the chronic radiation nephropathy. The second type involves epithelial nuclear atypia, such as crowding of the renal tubules by cells with nuclei two to three times the size of normal tubular epithelial cells and changes in chromatin distribution. The first studies of nuclear atypia in renal tubular epithelium have been described associated to collecting duct RCCs [11]. However, in our cases, we could not relate peritumoral nuclear atypia with histological type of RCC.

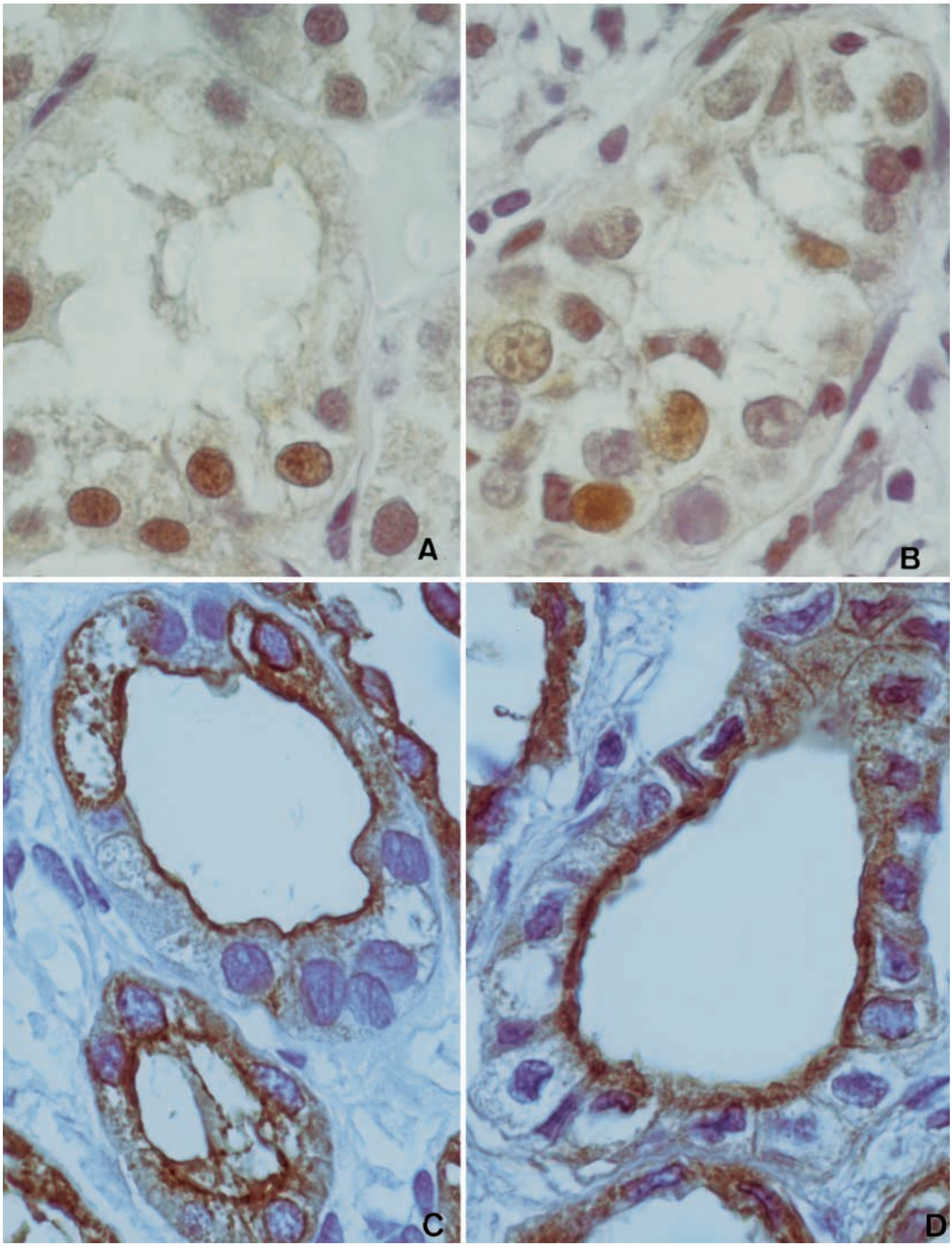
Well-known classic descriptions of acute and chronic radiation effects on the kidney do not include the pathology and pathogenesis of human renal injury after long-term (14 years) low-dose exposure of ionizing radiation. Nevertheless, the above-mentioned persistent chronic radiation nephropathy, from a morphologic viewpoint, could be presented as a sclerosing proliferative atypical nephrosis, as was observed in the majority of Ukrainian patients, including group I. As evidenced by low to moderate medullar epithelial nuclear atypia, with low to severe predominantly medullar irradiation lesions of peritumoral kidney in patients from group I, the socalled “clean” area in Ukraine is not completely clean, because the ground, water, and food could easily have been radiocontaminated, as was confirmed in our previous study [30].

The predominant regenerative and proliferative tubular and collecting duct changes of the radiation sclerosing proliferative atypical nephropathy (RSPAN) of Ukrainian patients were associated with a dramatic increase of PCNA and K-*ras* protein staining in comparison with the control Spanish group of analog patients.

The most striking feature was the significant increase of PCNA staining involving epithelial tubular nuclei of cortex and medullar collecting ducts in all Ukrainian groups (compared with PCNA minimal staining in the Spanish group). This was indicative not only of increased cell proliferation, but it could point to an essential role of PCNA in nucleic acid metabolism, as a component of the replication and repair machinery [16].

The role of oncogenes and, in particular, *ras* genes *(H-ras, N-ras, K-ras*) in the development of RCC has not yet been fully clarified. Recent studies suggest the *H-ras* and *K-ras* oncogene activation in RCC determine their possible involvement in renal-cell carcinogenesis [10, 33]. Some studies indicate that *K-ras* amplificaion might be essential to the proliferation, to a more rapid progression and aggressivity of human renal-cell carcinomas [17, 24, 33]. Numerous investigators have experimentally detected the expression of *ras*-oncogenes in radiation-induced tumors [19, 25]. Chronic long-term low-dose radiation inducing DNA damage with subsequent DNA repairing processes and permanent chronic proliferation of tubular and collecting ducts epithelium of peritumoral tissues in Ukrainian patients possibly activates *ras* family genes. A strong relationship between K-*ras* protein expression of atypical peritumoral lesions in association with increased blood vessel permeability, hemorrhage, and chronic radiation exposure of Ukrainian patients

Fig. 2 Immunohistochemical findings for peritumoral kidney tissues of Ukrainian patients exposed to long-term, low-dose ionizing radiation. **A**, **B** Cortical and medullar proliferating cell nuclear antigen (PCNA) expression. **C**, **D**, K-*ras* collecting ducts expression. Magnification ×400 ▶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confirms this possibility. Recent studies have shown the significant activation of angiogenesis modulated by *K-ras* activity that was induced in response to ionizing radiation [2, 8, 32]. These studies could explain the medullar changes of blood vessel permeability associated with K-*ras* expression of collecting duct epithelium of irradiated medulla in peritumoral kidney of Ukrainian patients, which are usually known to have an important prognostic value for RCCs [9, 49].

Recent studies also show that the target for the induction of persistent genomic instability effected by long-term low-dose ionizing radiation is not the DNA but more likely the cytoplasmic membrane due to the production of free radicals through oxidative processes [39, 40]. This means that the crucial initiating event is not directly caused by the interaction of radiation tracks with the DNA. In the kidney, which is a solid coupled tissue, the complicate gap junction intercellular communication (GJIC) is found to be the main target for low doses of ionizing radiation [38]. Radiation injury of GJIC appears to be critical in cells that appear to precede the onset of DNA synthesis and stimulate cell division [41]. It is interesting to note the potential role of the human *ras* oncogenes in the inhibition of GJIC between epithelial cells through a change in the phosphorylation of the major gap-junction protein [4, 43] and also the role of *ras* proto-oncogene in signal transduction involving the cytoskeleton of renal epithelial cells, leading to renal carcinogenesis and development of tumors of a more aggressive phenotype [1].

In conclusion, the present data point to a strong relationship between the long-term of low-dose radiation exposure of people living more than 14 years in radio-contaminated (^{137}C) areas of Ukraine and the development of chronic radiation nephropathy. Our data also confirm the possible initiator, promoter, or progressor role of chronic low-level radiation of renal human carcinogenesis in Ukraine.

Acknowledgements This study was supported by grants of Valencia University and by the Interministerial Commission of Science and Technology (CICYT), SAF 97/0205.

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