Sensitization of colorectal cancer cells to irradiation by IL-4 and IL-10 is associated with inhibition of NF-κB

R. VOBORIL[1,2*], J. WEBEROVA-VOBORILOVA[1**]

[1] *Department of Cell and Molecular Biology, Charles University 3rd Faculty of Medicine, Prague, Czech Republic*
[2] *Department of Surgery, Charles University 3rd Faculty of Medicine and Teaching Hospital Kralovske Vinohrady, Prague, Czech Republic, e-mail: rene.voboril@seznam.cz*

Received January 13, 2007

The efficiency of radiotherapy for rectal cancer treatment is limited because of radioresistance. Transcription factor nuclear factor-kappaB (NF-κB), which has antiapoptotic properties, may play an important role in this process. Recent studies indicate that behavior of the tumor is done not only by oncogenic events in tumor cells but also by microenvironment surrounding the tumor. Therefore we tested anti-inflammatory cytokines IL-4 and IL-10 occurring in tumor stroma whether they can modulate response of colorectal cancer cells to irradiation and whether this potential effect is associated with NF-κB. SW620 colorectal cancer cells were used for all experiments. Cell growth and clonogenicity were determined by cell proliferation assay and clonogenic assay, respectively. Activation of NF-κB was assessed by ELISA-based transcription factor assay and luciferase reporter assay. Apoptosis was determined by measuring caspase 3 activity. Irradiation (2–8 Gy) inhibited growth and clonogenicity of SW620 cells, induced apoptosis, and activated NF-κB, predominantly its subunits p50, p65, and RelB. IL-4 or IL-10 (1, 10, 100 ng/ml) neither inhibited growth and clonogenicity nor activated NF-κB, but they sensitized cells to irradiation in a dose dependent manner. Radiosensitization by IL-4 or IL-10 was associated with inhibition of NF-κB, predominantly its subunits p50 and p65 and increased apoptosis. In conclusion, modulation of the intestinal microenvironment, high local concentration of anti-inflammatory cytokines such as IL-4 and IL-10 may help to overcome resistance of colorectal tumors to radiotherapy. In this process NF-κB may be employed.

Key words: IL-4, IL-10, NF-κB, p65, radiosensitivity, colorectal cancer

Colorectal cancer is the second most common malignancy in developed countries, accounting for approximately 500,000 patients dying each year [1]. More than 30% of all cases are localized in the rectum. In advanced disease, radiotherapy represents one of important treatment modalities [2].

However, the efficiency of radiotherapy for rectal cancer treatment is limited. Cancer cells display different levels of intrinsic radiosensitivity and often develop secondary radioresistance [3, 4]. New approaches are required to overcome this radioresistance. It seems that inhibition of nuclear factor-kappaB (NF-κB) could be a promising way.

NF-κB is an inducible transcription factor that is involved in control of the expression of many genes including those important for apoptosis, cell cycle progression, and proliferation [5]. In most cells, NF-κB is located in the cytoplasm in the form of dimers composed of p50, p52, p65, c-Rel, and RelB subunits, and complexed with its inhibitor IκB [6]. After exposure to certain stimuli, including ionizing radiation or anticancer drugs, IκB is degraded and NF-κB is translocated into the nucleus to bind specific DNA sites [7]. However, NF-κB very often becomes constitutively activated in cancer cells of different origin including colorectal cancer [8, 9, 10, 11]. Moreover, activated NF-κB has been repeatedly shown to protect cancer cells from apoptosis [12, 13, 14]. Its inhibition enhanced apoptosis as well as sensitivity to anticancer treatment [15]. The best characterized subunit of NF-κB is p65 that forms together with p50 classical NF-κB heterodimer. This heterodimer is a potent activator of gene expression [16], where p65 is responsible for this activation [17]. Thus, it is supposed that p65 plays the key role in resistance to anticancer therapy.

* Corresponding author
** the authors contributed equally to this study

Recent reports indicate that properties of the tumor are done not only by oncogenic events in tumor cells but also by microenvironment surrounding the tumor. Microenvironmental signals coming predominantly from the tumor stroma may range from powerful tumoricidal effects to promotion of tumor growth [18]. These signals are mediated by number of mediators where interleukins, especially interleukin-4 (IL-4) and interleukin-10 (IL-10) are supposed to play very important role [19].

Human IL-4 has pleiotropic effects on a wide range of cell types [20]. In contrast to its growth stimulatory effect on lymphocytes, IL-4 has been described to have direct growth-inhibitory effect in vitro on breast cancer cells. Interestingly, IL-4 inhibited proliferation of breast cancer cells and had no effect on basal, unstimulated growth [21].

Human IL-10 has pleiotropic effects and even some contradictory properties [22]. It has been shown that IL-10 has positive as well as negative effect on tumor cell growth. For example, in thyroid cancer IL-10 seems to protect cancer cells from chemotherapy-induced apoptosis by upregulating the bcl-xL gene [23]. On the contrary, IL-10 could decrease the invasive potential of tumor cells by inhibiting matrix metalloproteinase secretion [24].

Both interleukins have been demonstrated to interact with NF-κB signaling pathway, inhibiting NF-κB activation subsequently after stimulation with LPS or TNF-α [25, 26]. However, to the best of our knowledge their sensitizing effect on cancer cells in combination with irradiation has not yet been studied. Our experiments were performed on human colorectal cancer cell line SW620 that has been previously shown to be the most radioresistant from the cell lines we tested, and with the high constitutive NF-κB activity as well [11].

The aims of the study were a) to assess the effect of IL-4 and IL-10 on sensitivity of colorectal cancer cells to irradiation, b) to determine if this potential effect is associated with inhibition of NF-κB, c) to identify if the only p65 subunit or other NF-κB subunits are employed in the potential inhibition of NF-κB, and d) to test whether IL-4 or IL-10 treatment combined with irradiation resulted in increased apoptosis.

Material and methods

Tumor Cell Line and Culture. The experiments were performed using the SW620 human colorectal cancer cell line. SW620 cells were cultured in RPMI 1640 medium (Sigma, St. Louis, MO, USA) supplemented with 10% heat-inactivated fetal bovine serum (Biochrom, Berlin, Germany) and antibiotics penicillin/streptomycin (Sigma, St. Louis, MO, USA). The cell culture was kept in a humified atmosphere of 5% CO_2 at 37°C.

Human recombinant interleukin-4 (IL-4) and interleukin-10 (IL-10) were purchased from Sigma (St. Louis, MO, USA). Each interleukin was administered 6 hours before irradiation and removed immediately after irradiation.

Cell Irradiation. SW620 cells were irradiated using Cobalt-60 source. Irradiation with doses of 2, 4, 6, or 8 Gy was administered at room temperature, and the cells were immediately placed back into the incubator. The control/sham-irradiated cells were maintained at the same conditions without irradiation.

Cell Proliferation Assay. Cell proliferation was determined by the colorimetric method using the CellTiter 96 AQueous One Solution Assay essentially as described by the manufacturer (Promega, Madison, WI, USA). Briefly, SW620 cells were plated in 96-well tissue culture plates, precultured with various doses of IL-4 or IL-10, and/or irradiated with 8 Gy. Cell proliferation was determined 72 h later during a 4 h pulse as described by the manufacturer.

Clonogenic Assay. Cells were precultured with interleukins and/or irradiated. Colonies were allowed to form in an undisturbed, humified environment with 5% CO_2 at 37°C. After 14 days, cells were fixed with 70% ethanol and stained with Coomassie Blue, and clones of at least 50 cells were counted as one colony. The surviving fraction was normalized to the surviving fraction of the corresponding control, and plotted as a function of dose on a log/linear plot.

Luciferase Reporter Assay. Cells were transfected using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) as described previously [11]. Twenty-four h after transfection cells were pre-cultured with interleukins and irradiated with 8 Gy. Luciferase activities were determined 24 h later using the dual-luciferase reporter assay system (Promega, Madison, WI, USA) and expressed as adjusted RLU.

Cell Extracts and DNA Binding Activity Assay for NF-κB. Two h after irradiation nuclear and cytoplasmic extracts were prepared using the NE-PER kit as described by the manufacturer (Pierce Biotechnology, Rockford, IL, USA). Protein concentrations were determined using the BCA Protein Assay Kit as described by the manufacturer (Pierce Biotechnology, Rockford, IL, USA).

Activation of the NF-κB subunits in 10 μg of SW620 nuclear extracts was determined using an NF-κB enzyme-linked immunosorbent assay (ELISA)-based transcription factor assay kit (TransAM Assay) (Active Motif, Carlsbad, CA, USA) according to the manufacturer's protocol. In brief, nuclear extract was added to each well of a 96-well plate into which an oligonucleotide with a NF-κB consensus binding site (5'-GGGACTTTCC-3') had been immobilized. The active form of NF-κB contained in the cell extract specifically binds to this oligonucleotide. After incubation, wells were washed and then incubated with primary antibodies against each NF-κB subunit, and secondary horseradish peroxidase-conjugated antibody. After incubation with developing solution, the reaction was stopped. Finally, the absorbance at 450 nm was read on ELISA microplate reader.

Caspase 3 Assay. Caspase 3 activity was determined by measuring the absorbance at 405 nm after cleavage of synthetic substrate Ac-DEVD-pNA using the Caspase 3 Assay Kit (Sigma, St. Louis, MO, USA) as described previously [11].

Briefly, SW620 cells were pre-cultured with interleukins and/or irradiated with 8 Gy. Forty-eight hours later cells were lysed and activity of caspase 3 was assessed.

Statistical Analysis. Data are expressed as mean values ± SEM. Differences between experimental groups were considered significant at a p value < 0.05 from Student's t-test.

Results

IL-4 and IL-10 treatment enhanced radiosensitivity of SW620 cells. We have previously shown that colorectal cancer cells SW620 are highly resistant to ionizing radiation [11]. To determine if IL-4 or IL-10 may reverse the radioresistance of colorectal cancer cells, SW620 cells were pretreated with various concentrations (1, 10, 100 ng/ml) of IL-4 or IL-10 for 6 hours, and/or irradiated with 8 Gy. After 72 hours of incubation, cell proliferation was measured (Fig. 1).

Irradiation with 8 Gy significantly (p = 0.028) reduced proliferation of SW620 cells in comparison to the control group. In contrast, treatment with increasing doses of IL-4 and IL-10 caused a slight but insignificant inhibition in the growth of SW620 cells. However, IL-4 and IL-10 enhanced growth inhibitory effect of irradiation in a dose dependent manner, but only the highest concentration (100 ng/ml) of both interleukins had significant enhancement effect on irradiation-caused cell growth inhibition (IL-4: p = 0.039; IL-10: p = 0.044) when compared to untreated irradiated cells. These data suggest that IL-4 and IL-10 are effective at augmenting the growth inhibition in combination with irradiation.

To further confirm radiosensitizing effect of IL-4 and IL-10, SW620 cells were pretreated with 100 ng/ml of IL-4 or IL-10 for 6 hours, and irradiated with 0, 2, 4, 6, and 8 Gy and assessed for clonogenic cell survival. Figure 2 shows that both interleukins suppressed the clonogenic survival of SW620 cells. Survival at 2 Gy was reduced by IL-4 treatment from 0.75 ± 0.04 to 0.51 ± 0.05 (p = 0.017) and by IL-10 treatment from 0.75 ± 0.04 to 0.59 ± 0.04 (p = 0.041). IL-4 and IL-10 alone did not influence clonogenic survival. Survival enhancement ratios were calculated at the 50% cell survival by dividing radiation dose of the radiation-only survival curve with that of the corresponding IL-4 or IL-10 plus radiation curve. Survival enhancement ratio was 1.26 for the IL-4 treated cells and 1.16 for the IL-10 treated cells.

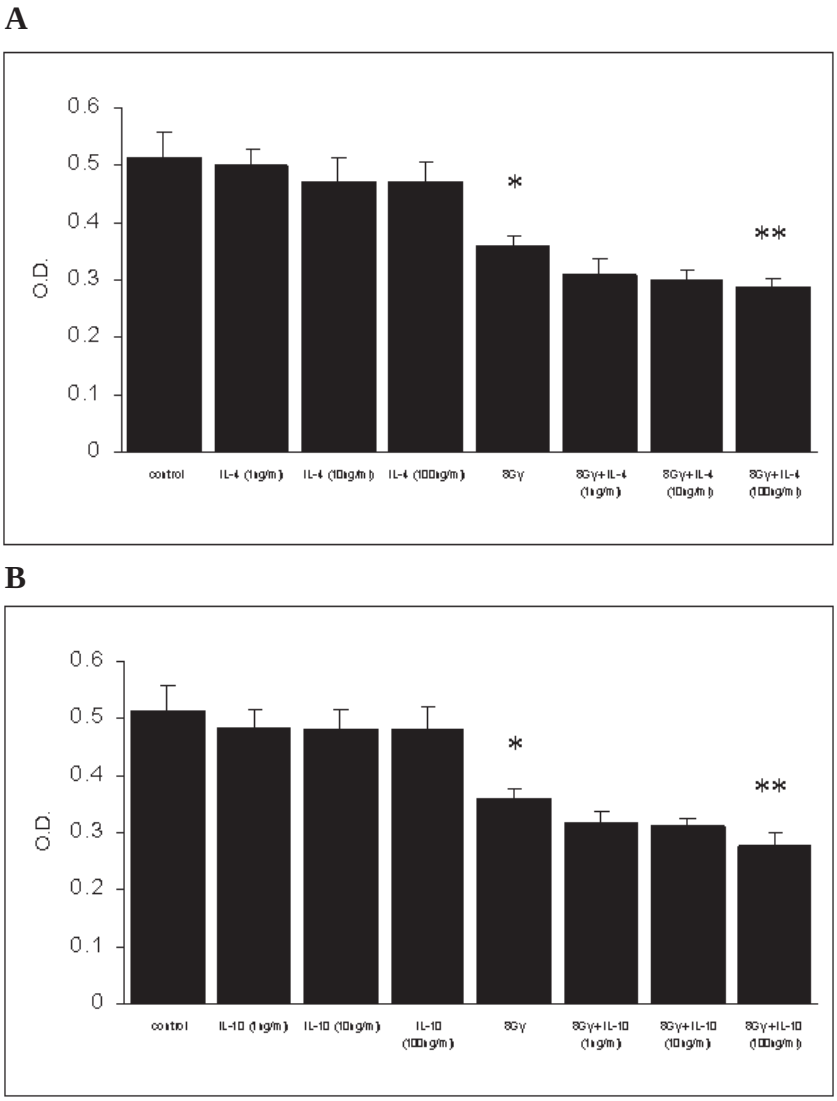

Figure 1. Treatment with IL-4 or IL-10 enhances radiation-induced growth inhibition in SW620 cells. SW620 cells were pretreated with IL-4 or IL-10 for 6 hours and/or irradiated with 8 Gy. Inhibition of growth was determined 72 h later. Treatment with (A) IL-4 or (B) IL-10 alone did not significantly inhibit the growth of SW620 cells (p = NS), whereas irradiation did (*p = 0.028 *versus* control). IL-4 or IL-10 enhanced growth inhibitory effect of irradiation in a dose dependent manner. However, only 100 ng/ml of IL-4 or IL-10 enhanced growth inhibitory effect of irradiation with statistical significance (IL-4: **p = 0.039 *versus* irradiated; IL-10: **p = 0.044 *versus* irradiated).

IL-4 and IL-10 treatment inhibited radiation-induced NF-κB activation, predominantly its subunits p50 and p65. NF-κB has been reported to be activated by ionizing radiation [27]. To determine whether pretreatment with either IL-4 or IL-10 is associated with inhibition of radiation-induced NF-κB activation, SW620 cells were pretreated with IL-4 or IL-10 (100 ng/ml) for 6 h, and exposed to irradiation at 8 Gy.

The luciferase reporter gene assay showed that irradiation at 8 Gy caused strong activation of NF-κB-mediated transcription in comparison with control cells (p = 0.0001) (Fig.

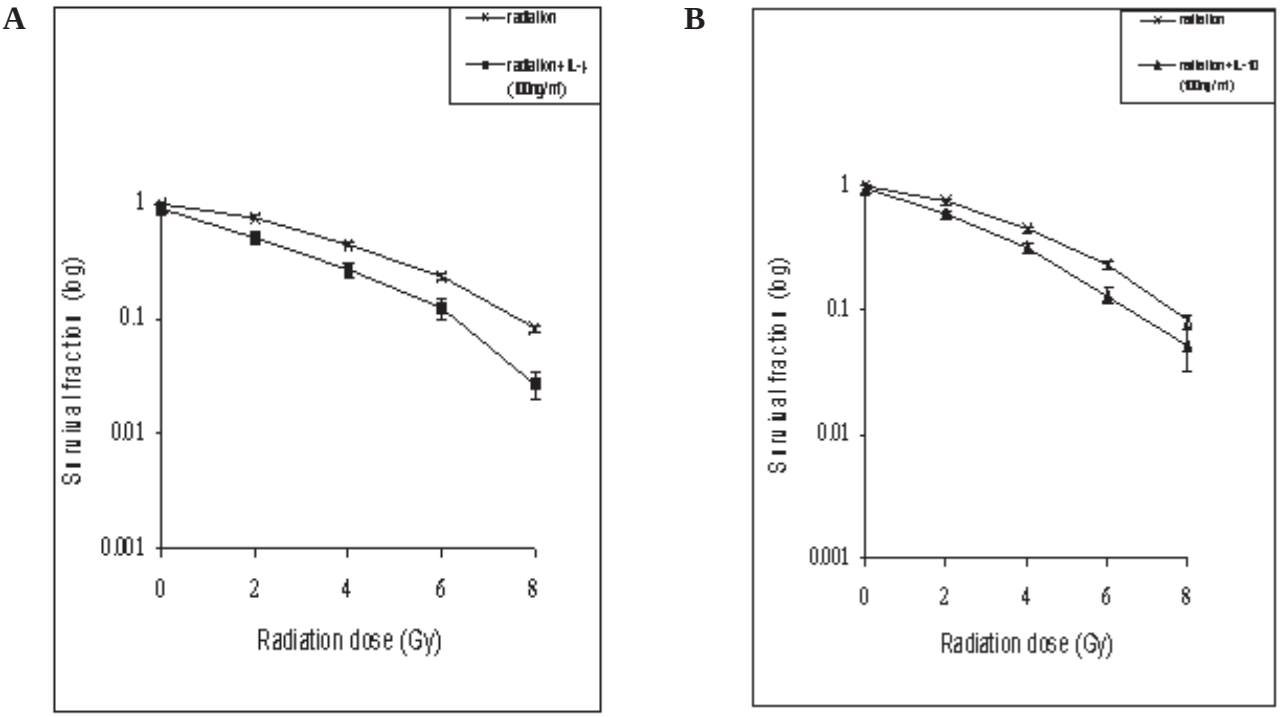

Figure 2. Treatment with IL-4 or IL-10 sensitizes SW620 cells to ionizing radiation. Radiosensitization by (A) IL-4 or (B) IL-10 was determined by clonogenic survival assays. SW620 were pretreated with IL-4 or IL-10 (100 ng/ml for 6 hours) and/or irradiated at various doses. After 14 days clonogenicity was assessed.

3). Treatment with various concentrations of IL-4 or IL-10 reduced radiation-induced activation of NF-κB-mediated transcription in a dose dependent manner (IL-4: 1 ng/ml p = 0.056, 10 ng/ml p = 0.001; 100 ng/ml p = 0.0008; IL-10: 1 ng/ml p = 0.67; 10 ng/ml p = 0.017; 100 ng/ml p = 0.001) (Fig. 3). IL-4 or IL-10 alone did not influence activation of NF-κB-mediated transcription.

Furthermore, we tested binding activity of all known NF-κB subunits (p50, p65, c-Rel, p52, and RelB) in nuclear extracts from SW620 cells using an NF-κB ELISA-based colorimetric assay. This method showed that irradiation caused increased binding activity of p50 (p = 0.00004), p65 (p = 0.0002), and RelB (p = 0.027) in comparison with control cells (Fig. 4A).

Combined treatment with IL-4 and irradiation caused significant decrease in NF-κB p50 subunit binding activity (p = 0.007) when compared to irradiated cells, and further decrease in p65 subunit (p = 0.039). Other NF-κB subunits RelB, c-Rel, and p52 also displayed NF-κB DNA binding inhibition that was determined as insignificant (Fig. 4B). IL-10 combined with irradiation resulted in significant decrease in NF-κB binding activity of p50 (p = 0.005) and p65 (p = 0.042). Furthermore inhibition of c-Rel, RelB, and p52 was recognized as insignificant (Fig. 4C). IL-4 or IL-10 alone did not influence binding activity of NF-κB subunits.

IL-4 and IL-10 treatment enhanced radiation-induced apoptosis as determined by caspase 3 activity. It has been shown that colorectal cancer cells displayed increased apoptosis after irradiation with gradual increasing from baseline to a maximum at 48 h after radiation treatment [28]. To test whether treatment with IL-4 or IL-10 enhanced radiation-induced apoptosis the cells were pretreated with either IL-4 or IL-10 (100 ng/ml) for 6 h and/or irradiated with 8 Gy. After 48 h the caspase 3 activity in cell extracts was measured using colorimetric caspase 3 assay. The results in Fig. 5 indicate that there was a minimal change in caspase 3 activity following IL-4 and IL-10 treatment only. After irradiation activity of caspase 3 in SW620 cells increased significantly (p = 0.0005) compared to control cells. Combination of IL-4 or IL-10 with 8 Gy resulted in further increase of caspase 3 activity in comparison with untreated irradiated cells (IL-4: p = 0.005; IL-10: p = 0.048).

Discussion

The present study was designed to evaluate the effects of IL-4 and IL-10 on the radiosensitivity of human colorectal cancer cells. Our results demonstrate that both interleukins, IL-4 and IL-10, enhanced radiosensitivity of SW620 cells. This radiosensitizing effect was associated with inhibition of

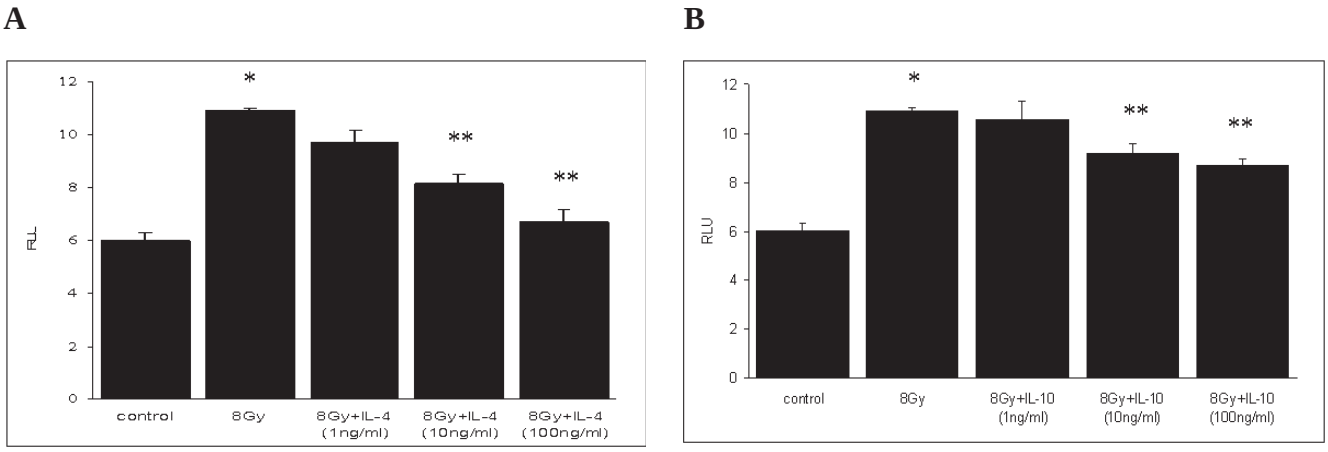

Figure 3. Irradiation causes activation of NF-κB-mediated transcription, which is inhibited by addition of either IL-4 or IL-10. The ability of IL-4 and IL-10 and/or irradiation of 8 Gy to induce NF-κB-dependent gene expression was determined by luciferase reporter gene assay. Firefly activities were corrected for Renilla activities and expressed as adjusted RLU. Irradiation caused strong NF-κB transcriptional activation in comparison with control cells (*p = 0.0001). Treatment with various concentrations of (A) IL-4 or (B) IL-10 reduced radiation-induced NF-κB transcriptional activation in a dose dependent manner (IL-4: 10 ng/ml **p = 0.001; 100 ng/ml **p = 0.0008) (IL-10: 10 ng/ml **p = 0.017; 100 ng/ml **p = 0.001). IL-4 or IL-10 alone did not influence NF-κB transcriptional activation (data not shown). ** depicts statistical significance as compared to irradiated cells. Positive and negative controls are not shown.

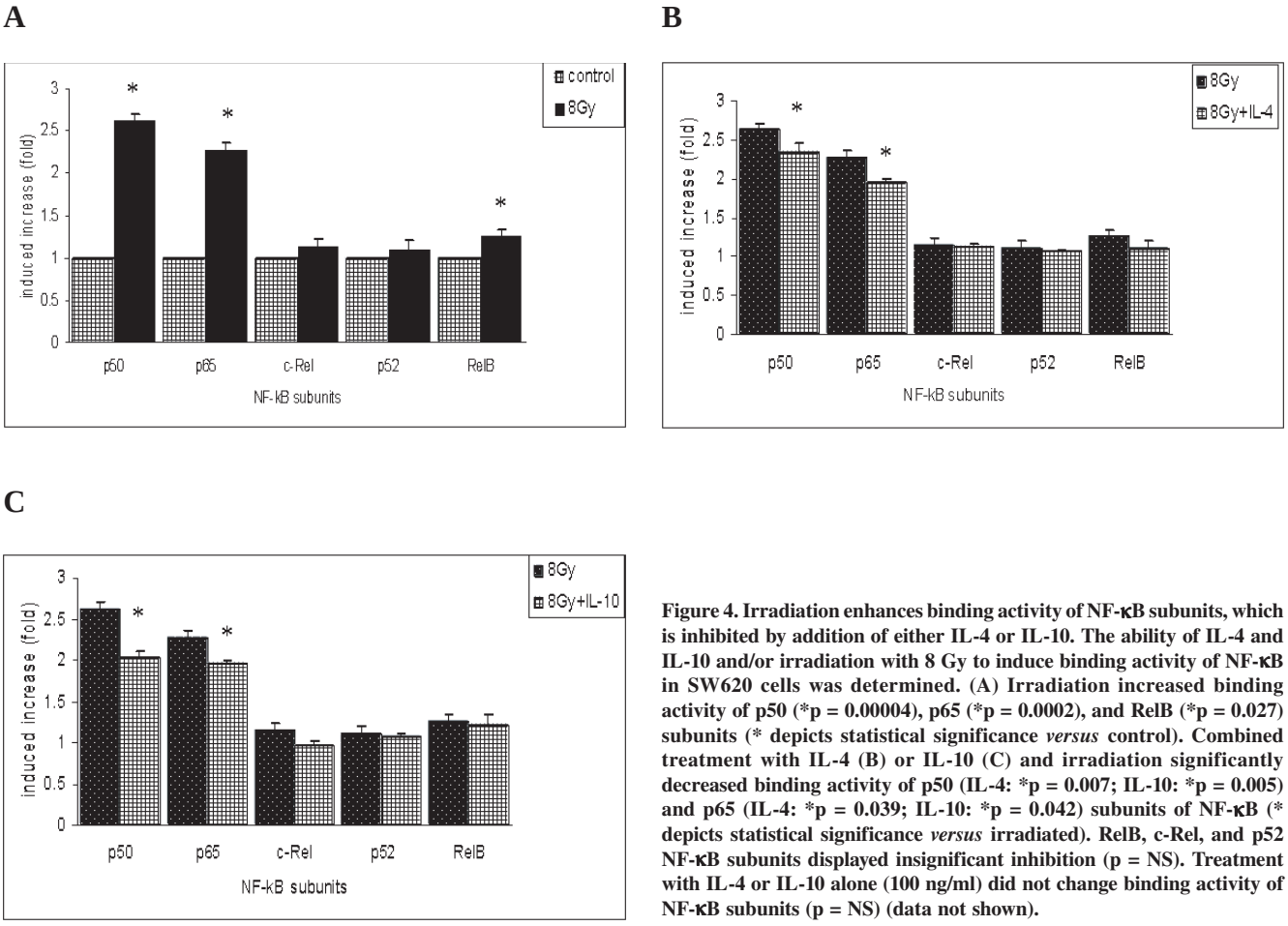

Figure 4. Irradiation enhances binding activity of NF-κB subunits, which is inhibited by addition of either IL-4 or IL-10. The ability of IL-4 and IL-10 and/or irradiation with 8 Gy to induce binding activity of NF-κB in SW620 cells was determined. (A) Irradiation increased binding activity of p50 (*p = 0.00004), p65 (*p = 0.0002), and RelB (*p = 0.027) subunits (* depicts statistical significance *versus* control). Combined treatment with IL-4 (B) or IL-10 (C) and irradiation significantly decreased binding activity of p50 (IL-4: *p = 0.007; IL-10: *p = 0.005) and p65 (IL-4: *p = 0.039; IL-10: *p = 0.042) subunits of NF-κB (* depicts statistical significance *versus* irradiated). RelB, c-Rel, and p52 NF-κB subunits displayed insignificant inhibition (p = NS). Treatment with IL-4 or IL-10 alone (100 ng/ml) did not change binding activity of NF-κB subunits (p = NS) (data not shown).

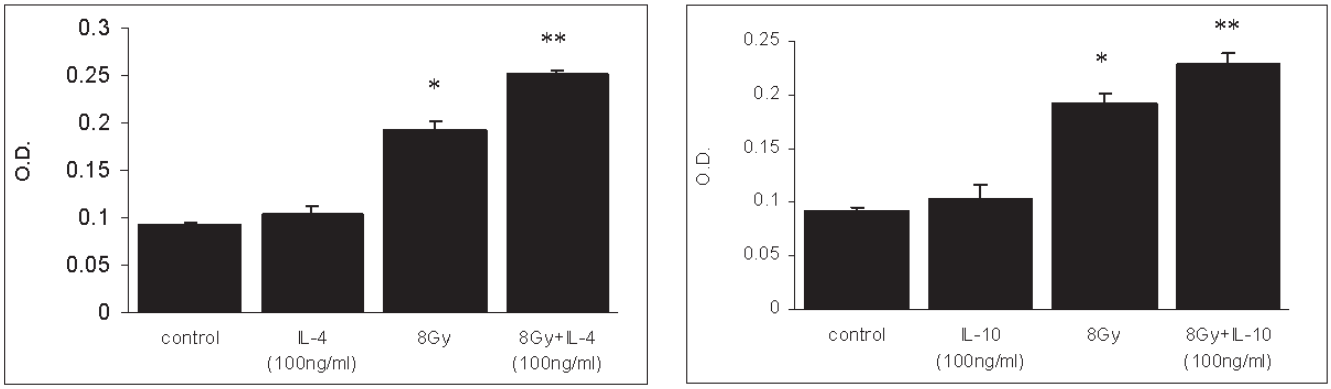

Figure 5. Inhibition of NF-κB by pretreatment with IL-4 or IL-10 significantly enhances radiation-induced apoptosis (caspase 3 activity) in SW620 cells. SW620 cells were treated for 6 h with (A) IL-4 or (B) IL-10 (100 ng/ml) and/or irradiated with 8 Gy. Caspase-3 activity was determined 48 hours later. Irradiation induced caspase 3 activity (*p = 0.0005 *versus* control) whereas IL-4 or IL-10 did not (p = NS). IL-4 or IL-10 further enhanced radiation-induced caspase 3 activity (IL-4: **p = 0.005; IL-10: **p = 0.048) (depicts statistical significance *versus* irradiated).**

NF-κB, predominantly p50 and p65 subunits, and increased apoptosis as determined by caspase 3 activity.

IL-4 and IL-10 belong to the family of anti-inflammatory cytokines. It is believed that both of them may contribute to tumor rejection by inhibition of stroma formation [19]. For example, IL-4 may inhibit participation of fibroblasts in tumor-induced angiogenesis [29]. Results of our study showed that IL-4 and IL-10 exert their inhibitory effect directly on cancer cells. This finding is supported by studies of other authors describing presence of receptors for IL-4 and IL-10 on the surface of cancer cells [21, 30, 31]. Moreover, we showed that these interleukins have not only inhibitory effect on proliferating cells as described previously [21] but they can also augment inhibitory effect of another inhibitor, in our case it was irradiation.

Results of a number of studies demonstrated a link between NF-κB and cancer [12, 14]. NF-κB as a transcription factor controls apoptosis, cell cycle progression and cell proliferation [32]. Radiation is one of known NF-κB activators [33] acting in a dose dependent manner [11]. Such activation can induce desensitization and radioresistance in cancer cells [34]. It has been shown that inhibition of NF-κB activation lead to increased radiosensitivity as described in experiments performed on cell lines. Expression of the IκB super-repressor or treatment with proteasome inhibitor PS-341 decreased radiation-induced NF-κB activation and resulted in an enhanced apoptotic response to radiation in colorectal cancer cells [28]. Overexpression of IκB sensitized human malignant glioma cells to radiation [35]. Similar results have been demonstrated in studies using for inhibition of radiation-induced NF-κB activation pharmacological agents such as indomethacin [36] or natural compounds curcumin [37] and genistein [38]. Therefore, there is a rationale for inhibition of activated NF-κB in tumor cells to become novel therapeutic strategy.

We have shown that IL-4 and IL-10 inhibit radiation-induced NF-κB activation. Previously, it has been demonstrated that these interleukins interact with NF-κB signaling pathway inhibiting NF-κB activation subsequently after stimulation with LPS or TNF-α [25, 26]. However, their possible radiosensitizing effect on cancer cells has not yet been described.

Most of the studies are focused on the whole NF-κB family or p65 subunit without further identification of subunits taking part in the process of activation. NF-κB exists in a form of homodimers or heterodimers composed from the subunits p50, p52, p65, c-Rel, and RelB. The classical NF-κB activation pathway known as *canonical* employs dimers composed predominantly from p65 and p50 subunits [39]. In prostate cancer, with the exception of c-Rel, significant nuclear expression of RelB, p65, p52, and p50 was seen in cancer cells. The *canonical* pathway dimers p50-p65 were less frequently observed than other subunit combinations such as RelB-p52 and RelB-p65 [40]. In endometrial carcinoma, immunohistochemical staining detected nuclear localization of all NF-κB subunits p50, p65, p52, c-Rel, and RelB [41]. In our experiments it was demonstrated that high constitutive NF-κB activation in SW620 cells is formed by all NF-κB subunits. Irradiation caused increased translocation of all NF-κB subunits, with predominant involving of p50, p65, and RelB, and minority involving of c-Rel and p52. Thus, we may consider that besides the *canonical* also another NF-κB activation pathway known as *alternative* may take place in this process. Pre-culture with both interleukins decreased radiation-induced NF-κB activation, IL-4 and IL-10 inhibited mainly p50 and p65, two of the most radiation-induced NF-κB subunits. Because both interleukins had radiosensitizing effect on SW620 cells we suggest that they exert their radiosensitizing effect through predominant inhibition of p50 and p65 subunits with minor inhibition of other NF-κB subunits RelB, c-Rel, and p52.

Results of this study may be of interest, because they may help to understand the processes influencing behavior of the tumor. Moreover, we hypothesize that modulation of the intestinal microenvironment; high local concentration of anti-inflammatory cytokines may help to overcome resistance of colorectal tumors to radiotherapy. These findings may have implications in the design of novel treatment strategies leading to enhancement of efficiency of radiation therapy. However, further investigation of IL-4 and IL-10 involvement in modification of the cellcs response to radiation is needed.

The study was supported by the grant of the Internal Grant Agency of the Ministry of Health of the Czech Republic No. NR/8053-3/2004.

References

[1] SCHNOLL-SUSSMAN F, MARKOWITZ AJ, WINAWER SJ. Screening and surveillance for colorectal cancer. Semin Oncol 2000; 27(Suppl 10): 10–21

[2] TAYLOR WE, DONOHUE JH, GUNDERSON LL, et. al. The Mayo Clinic experience with multimodality treatment of locally advanced or recurrent colon cancer. Ann Surg Oncol 2002; 9: 177–185

[3] LEITH J, FAULKNER G, PAPA P, et. al. In vitro radiation survival parameters of human colon tumour cells. Int J Radiat Oncol Biol Phys 1991; 20: 203–206

[4] CHAPMAN JD, ANDERSON PR. Predicting and overcoming the radioresistance of individual tumors. Int J Radiat Oncol Biol Phys 1999; 44: 477–479

[5] SEOK K, AGGARWAL BB. Transcription factor NF-κB. A sensor for smoke and stress signals. Ann NY Acad Sci 2005; 1056: 218–233

[6] KARIN M, BEN-NERIAH Y. Phosphorylation meets ubiquitination: the control of NF-κB activity. Annu Rev Immunol 2000; 18: 621–663

[7] BALDWIN AS Jr. The NF-κB and IκB proteins: new discoveries and insights. Annu Rev Immunol 1996; 14: 649–681

[8] BOURS V, DEJARDIN E, GOUJON-LETAWE F, et. al. The NF-kappa B transcription factor and cancer: high expression of NF- kappa B- and I kappa B- related proteins in tumor cell lines. Biochem Pharmacol 1994; 47: 145–149

[9] CUSACK JC JR, LIU R, BALDWIN AS Jr. Inducible chemoresistance to 7-ethyl-10-[4-(1-piperidino)-1-piperidino]-carbonyloxycamptothecin (CPT-11) in colorectal cancer cells and a xenograft model is overcome by inhibition of nuclear factor-kB activation. Cancer Res 2000; 60: 2323–2330

[10] LIND DS, HOCHWALD SN, MALATY J, et. al. Nuclear factor-kappaB is upregulated in colorectal cancer. Surgery 2001; 130: 363–369

[11] VOBORIL R, WEBEROVA-VOBORILOVA J. Constitutive NF-κB activity in colorectal cancer cells: impact on radiation-induced NF-κB activity, radiosensitivity, and apoptosis. Neoplasma, 2006; 53: 518–523.

[12] BOURS V, BENTIRES-ALJ M, HELLIN AC, et. al. Nuclear factor-κB, cancer, and apoptosis. Biochem Pharmacol 2000; 60: 1085–1090

[13] AGGARWAL BB. Apoptosis and nuclear factor-kB: a tale of association and dissociation. Biochem Pharmacol 2000; 60: 1033–1039

[14] LIN A, KARIN M. NF-κB in cancer: a marked target. Sem Cancer Oncol 2003; 13: 107–114

[15] VOBORIL R, HOCHWALD SN, LI J, et. al. Inhibition of NF-κB augments sensitivity to 5-fluorouracil/folinic acid in colon cancer. J Surg Res 2004; 120: 178–188

[16] KARIN M, CAO Y, GRETEN FR, LI ZW. NF-κB in cancer: from innocent bystander to major culprit. Nat Rev Cancer 2002; 2: 301–310

[17] GHOSH S, MAY MJ, KOPP EB. NF-κB and Rel proteins: Evolutionarily conserved mediators of immune responses. Annu Rev Immunol 1998; 16: 225–260

[18] OOSTERLING SJ, VAN DER BIJ GJ, MEIJER GA, et. al. Macrophages direct tumour histology and clinical outcome in a colon cancer model. J Pathol 2005; 207: 147–155

[19] BLANKENSTEIN T. The role of tumor stroma in the interaction between tumor and immune system. Curr Opin Immunol 2005; 17: 180–186

[20] PAUL WE. Interleukin-4: A prototypic immunoregulatory lymphokine. Blood 1991; 77: 1859–1870

[21] GOOCH JL, LEE A, YEE D. Interleukin 4 inhibits growth and induces apoptosis in human breast cancer cells. Cancer Res 1998; 58: 4199–4205

[22] VICARI AP, TRINCHIERI G. Interleukin-10 in viral disease and cancer: existing the labyrinth? Immunol Rev 2004; 202: 223–236

[23] STASSI G, TODARO M, ZERILLI M, et. al. Thyroid cancer resistance to chemotherapeutic drugs via autocrine production of interleukin-4 and interleukin-10. Cancer Res 2003; 63: 6784–6790

[24] STEARNS ME, RHIM J, WANG M. Interleukin 10 (IL-10) inhibition of primary human prostate cell-induced angiogenesis: IL-10 stimulation of tissue inhibitor of metalloproteinase-1 and inhibition of matrix metalloproteinase (MMP)-2/MMP-9 secretion. Clin Cancer Res 1999; 5: 189–196

[25] CLARKE CJP, HALES A, HUNT A, et. al. IL-10-mediated suppression of TNF-α production is independent of its ability to inhibit NFκB activity. Eur J Immunol 1998; 28: 1719–1726

[26] SCHOTTELIUS AJG, MAYO MW, SARTOR RB, et. al. Interleukin-10 signaling blocks inhibitor of kB kinase activity and nuclear factor kB DNA binding. J Biol Chem 1999; 274: 31868–31874

[27] CATALDI A, RAPINO M, CENTURIONE L, et. al. NF-kappaB activation plays an antiapoptotic role in human leukemic K562 cells exposed to ionizing radiation. J Cell Biochem 2003; 89: 956–963

[28] RUSSO SM, TEPPER JE, BALDWIN AS Jr, et. al. Enhancement of radiosensitivity by proteasome inhibition: implications for a role of NF-κB. Int J Radiat Oncol Biol Phys 2001; 51: 183–193

[29] KAMMERTOENS T, SCHULER T, BLANKENSTEIN T. Immunotherapy: target the stroma to hit the tumor. Trends Mol Med 2005; 11: 225–231

[30] OBIRI NI, HILLMAN GG, HAAS GP, et. al. Expression of high affinity interleukin-4 receptors on human renal cell car-

cinoma cells and inhibition of tumor cell growth in vitro by interleukin-4. J Clin Invest 1993; 91: 88–93

[31] NARUKE M, ABE Y, HATANAKA H, et. al. Interleukin-10 expression is correlated with growth fraction in human non-small cell lung cancer xenografts. Int J Oncol 2001; 18: 1213–1217

[32] ORLOWSKI RZ, BALDWIN AS Jr. NF-kappaB as a therapeutic target in cancer. Trends Mol Med 2002; 8: 385–389

[33] BRACH MA, HASS R, SHERMAN ML, et. al. Ionozing radiation induces expression and binding activity of the nuclear factor kB. J Clin Invest 1991; 88: 691–695

[34] BALDWIN AS Jr. Control of oncogenesis and cancer therapy resistance by the transcription factor NF-kappaB. J Clin Invest 2001; 107: 241–246

[35] YAMAGISHI N, MIYAKOSHI J, TAKEBE H. Enhanced radiosensitivity by inhibition of nuclear factor κB activation in human malignant glioma cells. Int J Radiat Biol 1997; 72: 157–162

[36] BRADBURY CM, MARKOVINA S, WEI SJ, et. al. Indomethacin-induced radiosensitization and inhibition of ionizing radiation-induced NF-κB activation in HeLa cells occur via a mechanism involving p38 MAP kinase. Cancer Res 2001; 61: 7689–7696

[37] CHENDIL D, RANGA R, MEIGOONI D, et. al. Curcumin confers radiosensitizing effect in prostate cancer cell line PC-3. Oncogene 2004; 23: 1599–1607

[38] RAFFOUL JJ, WANG Y, KUCUK O, et. al. Genistein inhibits radiation-induced activation of NF-κB in prostate cancer cells promoting apoptosis and G_2/M cell cycle arrest. BMC Cancer 2006; 6: 107–116

[39] BONIZZI G, KARIN M. The two NF-kappaB activation pathways and their role in innate and adaptive immunity. Trends Immunol 2004; 25: 280–288

[40] LESSARD L, BEGIN LR, GLEAVE ME, et. al. Nuclear localisation of nuclear factor-kappaB transcription factors in prostate cancer: an immunohistochemical study. Br J Cancer 2005; 93: 1019–1023

[41] PALLARES J, MARTINEZ-GUITARTE JL, DOLCET X, et. al. Abnormalities in the NF-κB family and related proteins in endometrial carcinoma. J Pathol 2004; 204: 569–577