Constitutive NF-κB activity in colorectal cancer cells: impact on radiation-induced NF-κB activity, radiosensitivity, and apoptosis*

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Constitutive NF-κB activity has been found in many cancer cells of different origin. In our study we focused on constitutive NF-κB activity and its impact on radiation-induced NF-κB activity, intrinsic radiosensitivity, and apoptosis. Using colorectal cancer cell lines (Caco-2, SW480, SW620) we demonstrated that each cell line expresses different level of constitutive NF-κB activity. Moreover, irradiation caused secondary NF-κB activation which differed in each cell line. The cell lines tested displayed also different intrinsic radiosensitivities as was determined by clonogenic assay, and different spontaneous and radiation-induced apoptosis determined by activity of caspase 3. Complex analysis of our results revealed that there was a strong correlation between constitutive NF-κB activity and radiation-induced NF-κB activity (r=0.835), the level of constitutive NF-κB activity predicted the level of secondary, radiation-induced NF-κB activity. Furthermore, SW620 cells with the highest level of constitutive NF-κB activity displayed the lowest radiosensitivity and the lowest level of spontaneous apoptosis; Caco-2 cells with almost undetectable level of constitutive NF-κB activity displayed the highest radiosensitivity and even highest level of spontaneous apoptosis. SW480 cells showed intermediate level of constitutive NF-κB activity, intermediate radiosensitivity and intermediate level of spontaneous apoptosis. Our data suggest that the level of constitutive NF-κB activity may predict radiosensitivity of colorectal cancer cells. Such prediction may allow the individualization of patient treatment by radiotherapy.

Key words: NF-kappaB, colorectal cancer, prediction, radiosensitivity, apoptosis

Colorectal carcinoma is one of the most common malignancies and a leading cause of cancer mortality in industrialized countries [1]. In advanced disease, which develops in almost 50% of patients with colorectal cancer, a multimodality treatment approach including radiation is utilized [2].

It is well known that the extent of the response to radiotherapy varies from tumor to tumor even of the same histopathologic origin, with some being more radioresponsive than others [3]. Such variation in radiosensitivity emphasizes the need for identifying predictive markers or assays of radiation responses. Possible prediction of radiosensitivity of individual tumors would make an important contribution to enhancement of the efectiveness of radiotherapy allowing treatments to be planned specificaly for the individual patient [4, 5].

It is known that cells submitted to ionizing radiation respond by activation of transcription factors such as nuclear factor-kappaB (NF-κB) as a part of the cell's autodefense mechanism [6, 7]. NF-κB is a family of ubiquitous transcription factors with five known members: p50/p105, p52/p100, RelA (p65), c-Rel and RelB [8]. NF-κB occurs as a dimer and in non-stimulated cells is bound to an inhibitor (IκB) and retained in the cytoplasm. Only when activated, NF-κB is released from IκB and translocated into the nucleus [9]. This activation leads to regulation of the expression of over 150 genes [10] and subsequent protein synthesis influencing cell proliferation and apoptosis [11].

Tumor cells including colorectal cancer cells express different levels of constitutive NF-κB activity [12–14]. Moreover, exposure of these cells to anticancer therapy including

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ionizing radiation augments NF-κB activation [15, 16]. Therefore, the role of NF-κB in cellular resistance to anticancer therapy has been extensively studied. It is supposed that NF-κB activation may give tumor cells survival advantage and further may confer resistance to anticancer therapy including irradiation protecting tumor cells from apoptosis by induction of anti-apoptotic genes [17–19].

In the present study, using colorectal cancer cell lines we focused on constitutive NF-κB activity and its impact on radiation-induced NF-κB activity, intrinsic radiosensitivity, and apoptosis.

Material and methods

Cell lines and culture. Human colonic adenocarcinoma-derived cell lines SW480, SW620 and Caco-2 were grown 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). All cell lines were kept in a humified atmosphere of 5% CO_2 at 37 °C.

Cell irradiation. Plated cells were irradiated using Cobalt-60 source with a dose rate of 0.97 Gy/min. 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.

Clonogenic assay. Cells were seeded into 60-mm culture dishes, allowed to attach overnight and irradiated. Colonies were allowed to form in an undisturbed, humified environment with 5% CO_2 at 37 °C. After 10–14 days, cells were fixed with 70% ethanol and stained with Coomassie Blue, and the colonies containing more than 50 cells were counted. 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.

Cell extracts and electrophoretic mobility shift assay. 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 in nuclear and cytoplasmic extracts were determined using the BCA Protein Assay Kit as described by the manufacturer (Pierce Biotechnology, Rockford, IL, USA). EMSAs were performed using the Gel Shift Assay System as described by the manufacturer (Promega, Madison, WI, USA). Double stranded consensus oligonucleotide (5' AGTTGAGGGGACTTTCCCAGGC 3') was 5'-end-labeled with [γ-P^{32}]-ATP (Amersham Pharmacia Biotech, Piscataway, NJ, USA). Unincorporated label was removed by passing the mixture through a NucTrap push column (Stratagene, La Jolla, CA, USA). Binding reactions contained 15 μg of nuclear protein. Following binding reaction the samples were subjected to 4% PAGE analysis at 4 °C and autoradiography. Densitometry of each band representing level of NF-κB activity was performed.

Luciferase reporter assay. Activation of NF-κB in cell lines studied was determined using the PathDetect In Vivo Signal Transduction Pathway cis-Reporting system as described by manufacturer (Stratagene, La Jolla, CA, USA). Briefly, cells were plated at a density of $1.5x10^5$ cells per well. Twenty-four h later cells were transfected, using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA), with pNF-κB-Luc reporter plasmid (Stratagene, La Jolla, CA, USA), which contains the NF-κB binding site driving the *Firefly* luciferase reporter gene. Cells were also transfected with the equivalent negative control plasmid (pCIS-CK), which contains the luciferase reporter gene but lacks *cis*-acting NF-κB binding elements. Cells were cotransfected with the *Renilla* luciferase reporter plasmid phRL-TK (Promega, Madison, WI, USA), which was used for normalization of transfection efficiencies. Twenty-four h after transfection cells were irradiated with various doses ranging from 0 to 8 Gy. Luciferase activities were determined 24 h later using the dual-luciferase reporter assay system (Promega, Madison, WI, USA). *Firefly* activities were corrected for *Renilla* activities and expressed as adjusted RLU.

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). Briefly, $1x10^7$ exponentially growing cells were irradiated. At the indicated time points, cells were pelleted by centrifugation and resuspended in a chilled cell lysis buffer and incubated on ice. The protein concentration was measured by using a BCA kit (Pierce Biotechnology, Rockford, IL, USA). Then, the lysates (100 μg) were reacted with Ac-DEVD-pNA in an assay buffer (20 mM HEPES, 2 mM EDTA, 0.1% CHAPS, 5 mM DTT, pH 7.4). The mixtures were mantained at 37 °C and the formations of p-nitroaniline were subsequently analyzed by ELISA Micro-plate Reader. The caspase 3 activity was calculated in μmol of p-nitroaniline released per min per μg of cell lysate.

Statistical analysis. Data are expressed as mean values ±SEM. A p value of <0.05 from Student's test was considered to be statistically significant. Correlations between variables were obtained using Spearman's correlation test.

Results

Sensitivity of colorectal tumor cells to irradiation determined by the clonogenic assay. The intrinsic radiosensitivity of the three colorectal cell lines was determined by the colony-forming assay. As shown in Figure 1, Caco-2 was the most sensitive cell line with a survival fraction at 2 Gy (SF_2: Mean±S.E.M) of 0.56±0.01. SW620 cell line was the most radioresistant with SF_2 0.77±0.03, and SW480 was of intermediate radiosensitivity (SF_2 0.65±0.04) from the cell lines tested.

Constitutive and radiation-induced NF-κB activity. Based on EMSA method and subsequent densitometry we found different levels of constitutive NF-κB binding activities as

well as radiation-induced NF-κB binding activities in each cell line tested. Almost no detectable level of constitutive NF-κB activity was found in the nuclei of unirradiated Caco-2 cells (Fig. 2). On the other hand, constitutive NF-κB activity in unirradiated cells was highest in the cell line SW620 and lower in SW480 cells.

Irradiation with 8 Gy induced NF-κB activity in all cell lines (Fig. 2). Comparing these cell lines, the highest level of radiation-induced NF-κB activity was seen in the cell line with the highest level of constitutive NF-κB activity (SW620). The lowest level of radiation-induced NF-κB activity was seen in Caco-2 cell line with the lowest level of constitutive NF-κB activity.

To further confirm if irradiation caused an increase in the activation of NF-κB, luciferase reporter assay, using a plasmid construct (pNF-κB-Luc) containing direct repeats of the binding sites for NF-κB, was performed on the three cell lines studied after irradiation with 0, 2, 4, 6 and 8 Gy. These experiments demonstrated that irradiation with increasing doses induced NF-κB-dependent transcription of the luciferase gene in a dose dependent manner (Fig. 3).

Spontaneous and radiation-induced apoptosis. Different basal activities of caspase 3 as measured by the colorimetric method were detected in the cell lines studied. The highest level of spontaneous caspase 3 activity was found in Caco-2 cells and the lowest level of spontaneous caspase 3 activity was found in SW620 cells, whereas SW480 cells displayed intermediate spontaneous activity of caspase 3 (Fig. 4). Irradiation with 2, 4, 6 and 8 Gy induced caspase 3 activity in the cells at 48 h postirradiation in a dose dependent manner (Fig. 4).

Correlation between constitutive and radiation-induced NF-κB activity. The correlation between constitutive NF-κB activity and radiation-induced NF-κB activity is plotted in Figure 5. There is a strong correlation (r=0.835) and where the cell line had the highest level of constitutive NF-κB activity, the radiation-induced NF-κB activities were also the highest in the cell lines studied. On the other hand, in the cell line with almost no detectable constitutive NF-κB activity, the radiation-induced NF-κB activities also stayed at the very low levels.

Correlation between constitutive NF-κB activity and intrinsic radiosensitivity. The correlation between constitutive NF-κB activity and intrinsic radiosensitivity as represented by SF_2 is plotted in Figure 6. There is a strong correlation between the level of constitutive NF-κB activity and the fraction of cells surviving exposure to 2 Gy (r=0.891).

Correlation between constitutive NF-κB activity and spontaneous apoptosis. The correlation between the constitutive NF-B activity and the level of spontaneous apoptosis as measured by caspase 3 activity is plotted in Figure 7. There is a strong inverse correlation between the level of constitutive NF-κB activity and spontaneous caspase 3 activity at 48 h (r=–0.956). Cell line with the highest level of constitutive NF-κB activity had the lowest level of caspase 3 activity and *vice versa*.

Discussion

Constitutive NF-κB activity has been found in many cancer cells of different origin [20, 21]. Constitutively activated NF-κB may be associated with several aspects of tumorigenesis including promoting cancer cell proliferation, preventing apoptosis, enhancing angiogenesis and metastasis [22]. An inverse correlation between androgen receptor status and constitutive activity of NF-κB was found [23]. Constitutive NF-κB activity may play a role in the progression of prostate cancer and contribute to prostate cancer cell survival following androgen withdrawal [24]. NAKSHATRI et al. [25] reported that loss of hormone independency and progression to a more progressive tumor phenotype is associated with constitutive NF-κB activity in breast cancer. Constitutive NF-κB activity is also required for proliferation and survival of Hodgkin's disease tumor cells [26].

Our study was focused on the problem of constitutive NF-κB activity and its possible relationship with radiation-induced NF-κB activity in colorectal cancer cell lines. Among the cell lines tested, we found that the level of constitutive NF-κB activity predicts the level of radiation-induced NF-κB activity. SW620 cell line had the highest level of constitutive NF-κB activity, the radiation-induced NF-κB activities after irradiation with 2 Gy and 8 Gy were also the highest in comparison with the other cell lines studied. On the other hand, in Caco-2 cell line with almost no detectable constitutive NF-κB activity, the radiation-induced NF-κB activities also stayed at the very low levels.

A number of reports have been published demonstrating that NF-κB participates in cellular response to ionizing radiation of tumor cells [27–29]. However, conclusions from these reports regarding relationship between NF-κB activity and intrinsic radiosensitivity are conflicting. YAMAGISHI et al [27] showed that the inhibition of NF-κB activity by overexpression of IκB enhances the cellular radiosensitivity in human glioblastoma cells. DIDELOT et al [28] showed that modulation of constitutive NF-κB activity by dexamethasone or by TNF-α influences baseline apoptosis and intrinsic radiosensitivity in head-and-neck cancer cells. On the other hand, PAJONK et al [29] showed that inhibition of NF-κB activity by the IκB super-repressor in a human prostate cancer cell line and in a Hodgkin's lymphoma cell line failed to alter the intrinsic radiosensitivity. They concluded from their experiments that radioactivation of NF-κB does not determine the intrinsic radiosensitivity of cancer cells [29].

In our experiments, we found that SW620 cell line, which had the highest level of constitutive NF-κB activity, was the most resistant to irradiation as determined by the clonogenic assay. Conversely, Caco-2 cells, which had the lowest level of constitutive NF-κB activity, were the most sensitive to irradiation. There was found a strong correlation (r=0.891). We suggest that the level of constitutive NF-κB activity may predict radiosensitivity of colorectal cancer cells.

NF-κB suppresses apoptosis by inducing expression of a

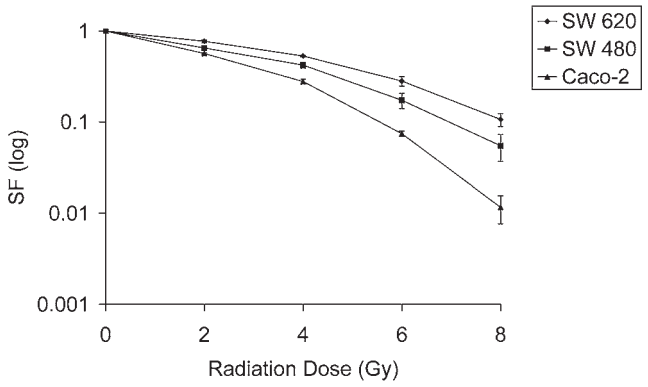
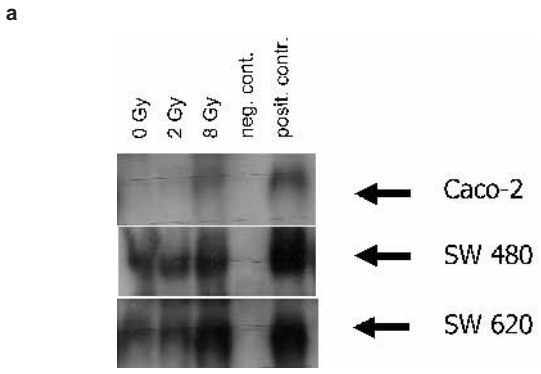


Figure 1. Cell survival of three colorectal cell lines as determined by the clonogenic assay (SF = surviving fraction). Points are means of three independent experiments ±SEM.



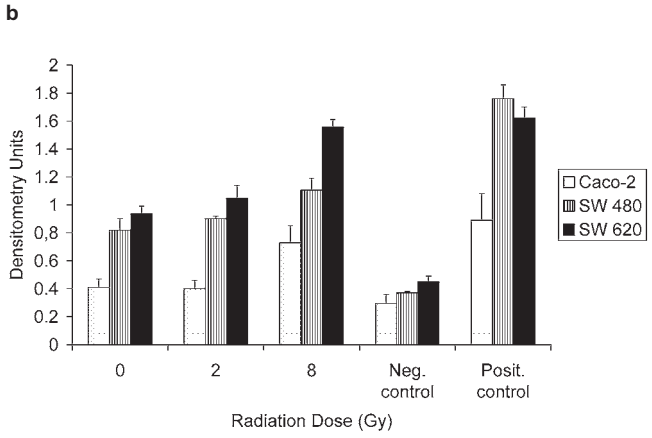
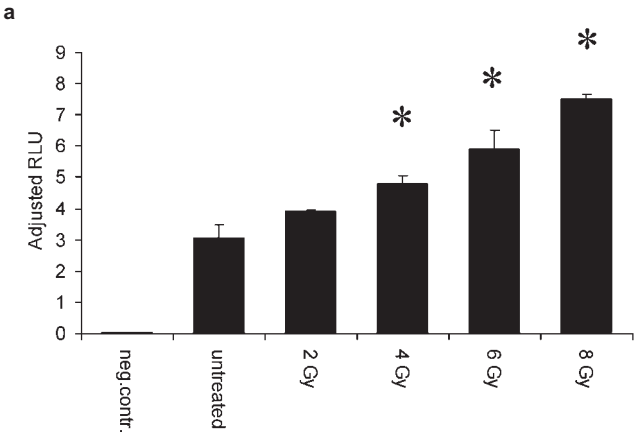
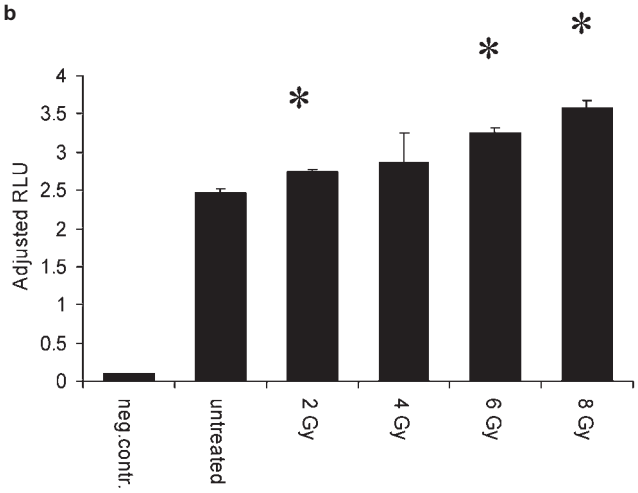


Figure 2. EMSA demonstrating nuclear NF-κB activity of three colorectal cancer cell lines. (a) Nuclear extracts from SW620, SW480 and Caco-2 cells prepared 2 h after irradiation with 0 (control group), 2 and 8 Gy are shown. Lane marked positive control represents the cells treated for 2 h with 10 ng/ml TNF-α. Lane marked negative control represents nuclear extracts from untreated cells competed with cold unlabeled NF-κB consensus oligonucleotide in the binding reaction. (b) The densitometric analysis of the bands seen in the upper panel.





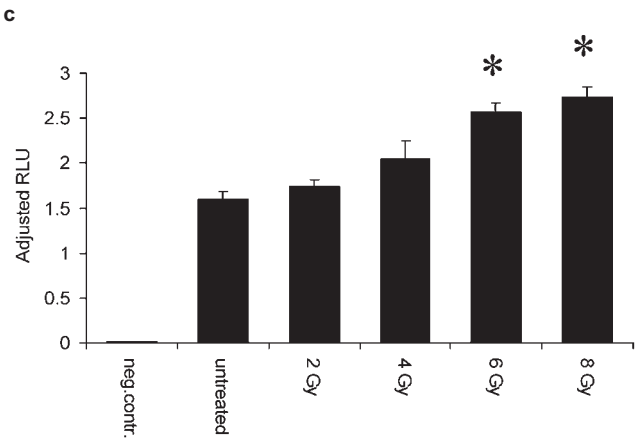


Figure 3. Luciferase Reporter Gene Assay of NF-κB-mediated transcription in three colorectal cancer cell lines (a) Caco-2, (b) SW480, and (c) SW620. Irradiation causes dose dependent activation of NF-κB-mediated transcription. The ability of each dose of irradiation to induce NF-κB-dependent gene expression was determined by Luciferase Reporter Gene Assay. Firefly activities were corrected for Renilla activities and expressed as adjusted RLU. *depicts significance as compared to untreated control (untreated control = 0 Gy), $p<0.05$.

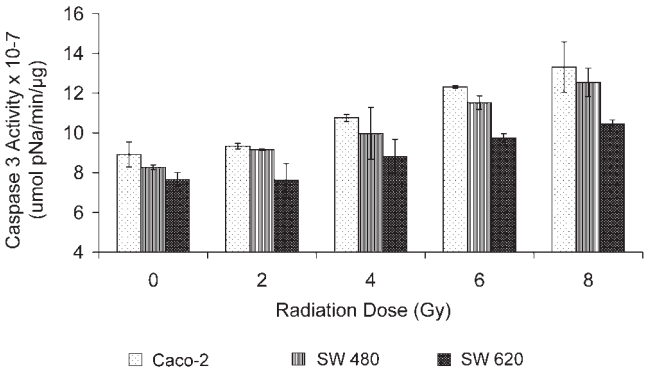


Figure 4. Spontaneous and radiation-induced apoptosis in three colorectal cancer cell lines of different intrinsic radiosensitivities. The level of apoptosis was determined by the measurement of caspase 3 activity 48 h after irradiation with 0 Gy (control group), 2, 4, 6 and 8 Gy. The caspase 3 activity is calculated in µmol of p-nitroaniline released per min per µg of cell lysate (pNA=p-Nitroaniline).

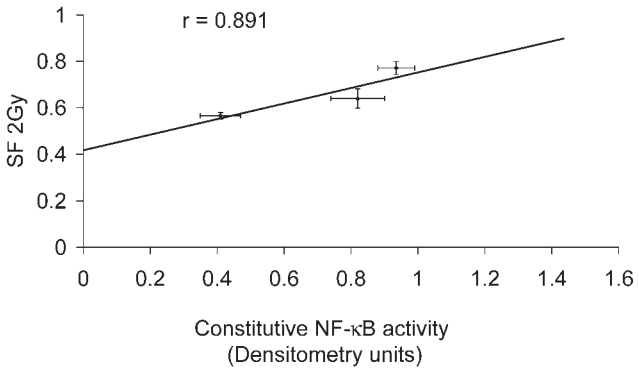


Figure 6. Correlation between constitutive NF-κB activity and intrinsic radiosensitivity as represented by surviving fraction after irradiation with 2 Gy (SF_2) of three colorectal cancer cell lines tested (r=0.891).

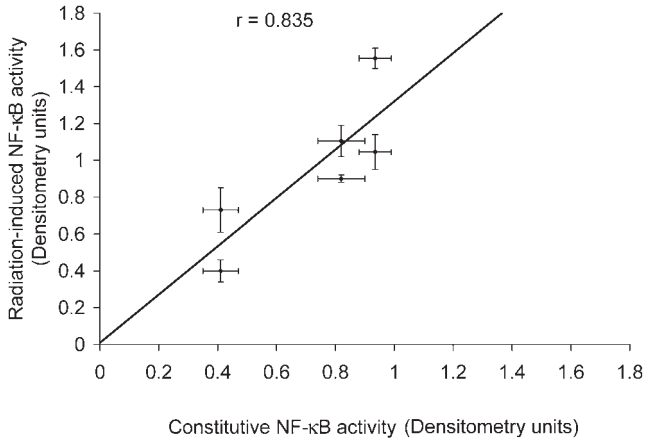


Figure 5. Correlation between constitutive NF-κB activity and radiation-induced NF-κB activity after irradiation with 2 and 8 Gy (r=0.835).

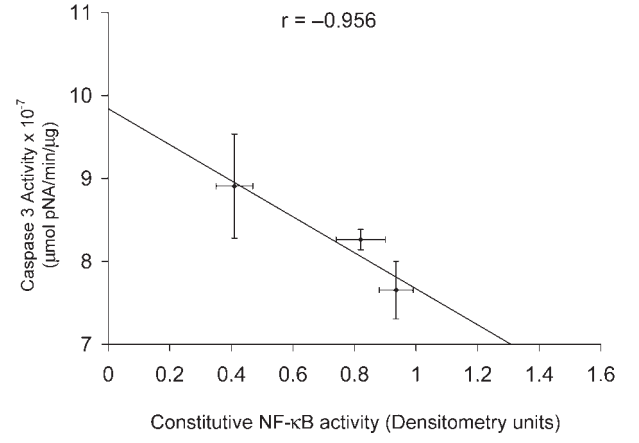


Figure 7. Correlation between constitutive NF-κB activity and spontaneous apoptosis as measured by caspase 3 activity in three colorectal cancer cell lines tested (r=–0.956).

number of genes whose products inhibit apoptosis, including inhibitors of apoptosis (IAPs) [19]. Their antiapoptotic activity seems to be related to ability to inhibit caspases, either by direct binding and inhibiting caspase 3 and caspase 7 or by preventing activation of pro-caspase 6 and pro-caspase 9 [30–32]. These findings have been confirmed in our study where the constitutive NF-κB activity was in a strong inverse correlation with the basal activity of caspase 3 (r=–0.956). We showed that the cell line with the highest level of constitutive NF-κB activity had the lowest rate of basal and radiation-induced activity of caspase 3 and *vice versa*. Thus, we suppose that NF-κB by protecting cells against apoptosis may give tumor cells an advantage of survival and confer them certain level of radioresistance.

This study demonstrates that the level of constitutive NF-κB activity is in a relationship with the intrinsic radiosensitivity of the colorectal cancer cell lines SW480, SW620 and Caco-2. Therefore, based on these results we suggest that constitutive NF-κB activity may play a central role in intrinsic radiosensitivity of tumor cells. From results of our study we conclude that NF-κB could serve not only as a target for novel and selective anticancer treatment strategies as described previously [33] but also as a prediction factor of radiation response of tumor cells. Such prediction may allow the individualization of patient treatment by radiotherapy.

Ultimately, applicability of the constitutive NF-κB activity as a predictive marker of response to irradiation in tumor cells may only be fully established by testing a wide range of cell lines and human tumor tissue samples.

References

[1] GREENLEE RT, HILL-HARMON MB, MURRAY T, THUN M. Cancer statistics 2001. CA Cancer J Clin 2001; 15–36.

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

[3] LEITH J, FAULKNER G, PAPA P, MICHELSON S. 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] GUO GZ, SASAI K, OYA N, SHIBATA T, SHIBUYA K, HIRAOKA M. A significant correlation between clonogenic radiosensitivity and the simultaneous assessment of micronucleus and apoptotic cell frequencies. Int J Radiat Biol 1999; 75: 857–864.

[6] LI N, KARIN M. Ionizing radiation and short wavelength UV activate NF-κB trough two distinct mechanisms. Proc Natl Acad Sci USA 1998; 95: 13012–13017.

[7] SHISHODIA S, AGGARWAL BB. Nuclear factor-κB: a friend or a foe in cancer? Biochem Pharmacol 2004; 68: 1071–1080.

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

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

[10] PAHL HL. Activators and target genes of Rel/NF-kappaB transcription factors. Oncogene 1999; 18: 6853–6866.

[11] 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.

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

[13] 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-κB activation. Cancer Res 2000; 60: 2323–2330.

[14] BOURS V, DEJARDIN E, GOUJON-LETAWE F, MERVILLE MP, CASTRONOVO V. The NF-kappaB transcription factor and cancer: high expression of NF-kappaB- and I-kappaB- related proteins in tumor cell lines. Biochem Pharmacol 1994; 47: 145–149.

[15] WANG CY, MAYO M, BALDWIN AS. TNF- and cancer therapy-induced apoptosis: potentiation by inhibition of NF-κB. Science 1996; 274: 784–787.

[16] CUSACK JC JR. Overcoming antiapoptotic responses to promote chemosensitivity in metastatic colorectal cancer to the liver. Ann Surg Oncol 2003; 10: 852–862.

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

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

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

[20] WANG W, ABBRUZZESE JL, EVANS DB, LARRY L, CLEARY KR, CHIAO PJ. The nuclear factor-kappaB RelA transcription factor is constitutively activated in human pancreatic adenocarcinoma cells. Clin Cancer Res 1999; 5: 119–127.

[21] DEJARDIN E, DEREGOWSKI V, CHAPELIER M, JACOBS N, GIELEN J et al. Regulation of NF-kappaB activity by IkappaB-related proteins in adenocarcinoma cells. Oncogene 1999; 18: 2567–2577.

[22] BHARTI AC, AGGARWAL BB. Nuclear factor-kappaB and cancer: its role in prevention and therapy. Biochem Pharmacol 2002; 64: 883–888.

[23] SUH J, PAYVANDI F, EDELSTEIN LC, AMENTA PS, ZONG WX et al. Mechanisms of constitutive NF-kappaB activation in human prostate cancer cells. Prostate 2002; 52: 183–200.

[24] SUH J, RABSON AB. NF-kappaB activation in human prostate cancer: important mediator or epiphenomenon? J Cell Biochem 2004; 91: 100–117.

[25] NAKSHATRI H, BAT-NAKSHATRI P, MARTIN DA, GOULET RJ JR, SLEDGE GW JR. Constitutive activation of NF-kappaB during progression of breast cancer to hormone-independent growth. Mol Cell Biol 1997; 17: 3629–3639.

[26] BARGOU RC, EMMERICH F, KRAPPMANN D, BOMMERT K, MAPARA MY et al. Constitutive nuclear factor-κB-RelA activation is required for proliferation and survival of Hodgkin's disease tumor cells. J Clin Invest 1997; 100: 2961–2969.

[27] 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.

[28] DIDELOT C, BARBERI-HEYOB M, BIANCHI A, BECUWE P, MIRJOLET JF et al. Constitutive NF-κB activity influences basal apoptosis and radiosensitivity of head-and-neck carcinoma cell lines. Int J Radiation Oncology Biol Phys 2001; 51: 1354–1360.

[29] PAJONK F, PAJONK K, MCBRIDE WH. Inhibition of NF-κB, clonogenicity, and radiosensitivity of human cancer cells. J Natl Cancer Inst 1999; 91: 1956–1960.

[30] WANG CY, MAYO MW, KORNELUK RG, GOEDDEL DV, BALDWIN AS JR. NF-kappaB antiapoptosis: induction of TRAF1 and TRAF2 and c-IAP1 and c-IAP2 to suppress caspase-8 activation. Science 1998; 281: 1680–1683.

[31] DEVERAUX QL, ROY N, STENNICKE HR, VAN ARSDALE T, ZHOU Q et al. IAPs block apoptotic events induced by caspase-8 and cytochrome c by direct inhibition of distinct caspases. EMBO J 1998; 17: 2215–2223.

[32] CHU ZL, MCKINSEY TA, LIU L, GENTRY JJ, MALIM MH, BALLARD DW. Suppression of tumor necrosis factor-induced cell death by inhibitor of apoptosis c-IAP2 is under NF-κB control. Proc Natl Acad Sci USA 1997; 94: 10057–10062.

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