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Transcriptional Reprogramming Regulates Tumor Cell Survival in Response to Ionizing Radiation: a Role of p53

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Senexin B, a non-toxic selective inhibitor of cyclin-dependent protein kinases 8 and 19 (CDK8 and CDK19), in combination with γ-photon irradiation in doses of 2-10 Gy increased the death of colon adenocarcinoma cell line HCT116 (intact p53) in a logarithmically growing culture, which was accompanied by the prevention of cell cycle arrest and a decrease of "senescence" phenotype. The effect of senexin B in cells with intact p53 is similar to that of *Tp53* gene knockout: irradiated HCT116p53KO cells passed through the interphase and died independently of senexin B. The inhibitor reduced the ability of cells to colony formation in response to irradiation; p53 status did not affect the effectiveness of the combination of radiation and senexin B. Thus, the CDK8/19 inhibitor senexin B increased cell sensitivity to radiotherapy by mechanisms dependent and independent of p53 status.

Key Words: *transcriptional reprogramming; ionizing radiation; cyclin-dependent protein kinases 8/19; p53; cell death*

Transcriptional reprogramming is a mechanism of optimization of gene expression for adaption of cells to stress. This mechanism is mediated by cyclin-dependent protein kinases 8 and 19 (CDK8/19) in complexes with cyclin C, MED12, and MED13, thereby regulating the traverse of RNA polymerase II along the DNA template [1]. CDK8/19 are functional only in specific contexts, that is, inducible (but not basal/constitutive) transcription is reprogrammed in response to specific stimuli. Among the prerequisites for CDK8/19-mediated transcriptional reprogramming are activation of transcription factor NF-κB in blood cells exposed to chemokines [2] and signaling cascades triggered by epidermal growth factor and estrogens [3,4]. Low-molecular-weight selective CDK8/19 inhibitors attenuated or abrogated the effects of the above-mentioned factors. In particular, they prevented activation of some cytokine genes and development of antitumor drug resistance [5-9].

The transcriptional factor p53 is a major sensor of DNA damage by a variety of stimuli including ionizing radiation. Accumulation of p53 in response to DNA damage initiates many signaling pathways leading to cell cycle disturbances and/or cell death. The p53 status (wild type of a non-functional mutant) is critical for the outcome of irradiation: p53 dysfunction prevents interphase cell arrest and repair of damage. Cells do not pass the S-G_2/M boundary and die in mitosis or after its completion [10]. In contrast, cells with the wild type p53 undergo G_2/M arrest and can survive. These cells frequently acquire a senescence-like phenotype: deceleration of RNA and protein synthesis and low proliferation rate, cell flattening and granularity, karyotypic abnormalities, accumulation of lipofuscin and activation of β-galactosidase [11]. Cells survived after single irradiation can generate a radioresistant population. In light of this, a question arises whether the sensitivity of tumor cells to radiotherapy can be

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improved by combining irradiation with inhibition of transcription reprogramming and whether the effectiveness of CDK8/19 inhibition depends on p53 status.

Thus, the aim of this work was to study the possibility of increasing the sensitivity of tumor cells to ionizing radiation upon inhibition of CDK8/19.

MATERIALS AND METHODS

Cell lines and culture conditions. The HCT116 human colon adenocarcinoma cell line (wild type p53) was purchased from the collection of Institute of Cytology, Russian Academy of Sciences. The isogenic p53 negative HCT116p53KO subline has been generated in B. Vogelstein Laboratory at Johns Hopkins University, Baltimore, MD; gift of B. P. Kopnin). The cells were cultured in DMEM supplemented with 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin (PanEco) at 37°C and 5% CO_2 in a humidified atmosphere. The selective CDK8/19 inhibitor senexin B (SnxB; produced by Senex Biotechnology, gift of I. Roninson, University of South Carolina, USA) [2] was added to cell cultures 3 h prior to irradiation. SnxB (1 μM) was present in cell cultures only during irradiation.

Cell irradiation. Cells in 25-cm^2 flasks (Eppendorf; 50% monolayers) were irradiated with γ-photons on a RUM-17 device (S. M. Kirov Military Medical Academy) using the following parameters: tube voltage 180 kV, current 10 mA, focus dimension 50 cm, filter 1 mm Al, 0.5 mCu, exposure rate 0.32 Gy/min, single doses 2-10 Gy. The dosimeter ID-11 and GO-32 device were used to measure the absorbed dose. Cell survival was tested in a logarithmically growing mass culture as well as by colony formation.

Analysis of cell viability in mass culture (MTT assay) and flow cytometry. Cell viability in mass culture 72 h after irradiation was assessed by MTT reduction. For flow cytometry, cells after irradiation were seeded to 60-mm Petri dishes (2×10^5/dish) in the presence or absence of 1 μM SnxB, incubated at 37°C and 5% CO_2 for 72 h, and then resuspended in lysis buffer (0.1% sodium citrate, 0.3% NP-40, 50 μg/ml RNase A (PanEco), 50 μg/ml PI (BD Pharmingen)). The cell cycle distribution (DNA ploidy) was analyzed on a CytoFlex B2-R2-V0 flow cytometer (Beckman Coulter) in PE or Rhodamine channels (20,000 events were accumulated for each sample). A 2D PE-W *vs* PE-A plot was used to exclude cell conglomerates.

Single cell survival (colony formation assay). Two hundred cells were plated in 100 mm Petri dishes, 10 ml of culture medium was added, and cells were incubated at 37°C, 5% CO_2 for 14 days in the same medium. Colonies were fixed with 10% methanol in saline, stained with 0.5% solution of crystal violet, and counted using gel documentation system ChemiDoc Touch (Bio-Rad). The number of colonies produced by non-irradiated cells was taken as 100%.

Cytochemical detection of β-galactosidase. To detect senescence associated β-galactosidase (SA-β-Gal), cells were fixed in 2% formaldehyde and 0.2% glutaraldehyde and stained in a buffer containing 40 mM citric acid/Na_2HPO_4 (pH 6.0), 5 mM $K_3Fe(CN)_6$, 5 mM $K_4Fe(CN)_6$, 0.15 M NaCl, 2 mM $MgCl_2\times6H_2O$ (PanEco) and 2.5 mM bromochloroindolylgalactopyranoside (X-Gal, Sigma-Aldrich) for 48-72 h at 37°C. In random fields of view, 100 cells were counted and the percentage of SA-β-Gal^+ cells was calculated.

Statistics. The data were processed statistically using Microsoft Excel 2016 and presented as $M\pm SD$. Significance of differences was evaluated using one-way ANOVA considered significant at $p\leqslant0.05$.

RESULTS

Survival of cells with different p53 status after irradiation and CDK8/19 inhibition. In preliminary experiments we found that incubation with 1 μM SnxB for 14 days did not affect doubling time of HCT116 and HCT116p53KO cells. Hence, SnxB was not toxic in our experiments and can be used in combination with other treatments. We analyzed the role of transcriptional reprogramming in survival of irradiated cells with different p53 status. To this end, HCT116 and HCT116p53KO cells were irradiated in single doses of 4 or 10 Gy (therapeutic range) with or without SnxB. In HCT116 cells exposed to 4 Gy, apoptotic signs, such as an extensive blebbing of the plasma membrane, cell shrinkage, nuclear condensation and fragmentation were detected 72 h after irradiation. In the presence of SnxB these changes were more pronounced (Fig. 1, *a*). Irradiation in a dose of 10 Gy induced even more pronounced alterations, while after exposure to 10 Gy in the presence of SnxB practically all cells were severely damaged. In contrast, irradiation of the HCT116p53KO subline was detrimental independently of SnxB (Fig. 1, *a*). Morphological signs correlated with cell survival: SnxB increased the radiation-induced death of HCT116 cells, but not HCT116p53KO cells. In 72 h after irradiation in doses of 4 and 10 Gy, MTT reduction by HCT116 cells was 67 and 43%, respectively. In HCT116p53KO cells, the corresponding values were 46 and 22% (Fig. 1, *b*). Thus, SnxB sensitized HCT116 cells to γ-photons, but had no effect on survival of irradiated HCT116p53KO cells. These results demonstrate the important role of transcriptional reprogramming and p53 in survival of irradiated cells.

Cell cycle distribution. Analysis of cell cycle distribution revealed significant differences in the mechanisms of the response of cells with different p53 status to irradiation and SnxB. In 72 h after irradiation

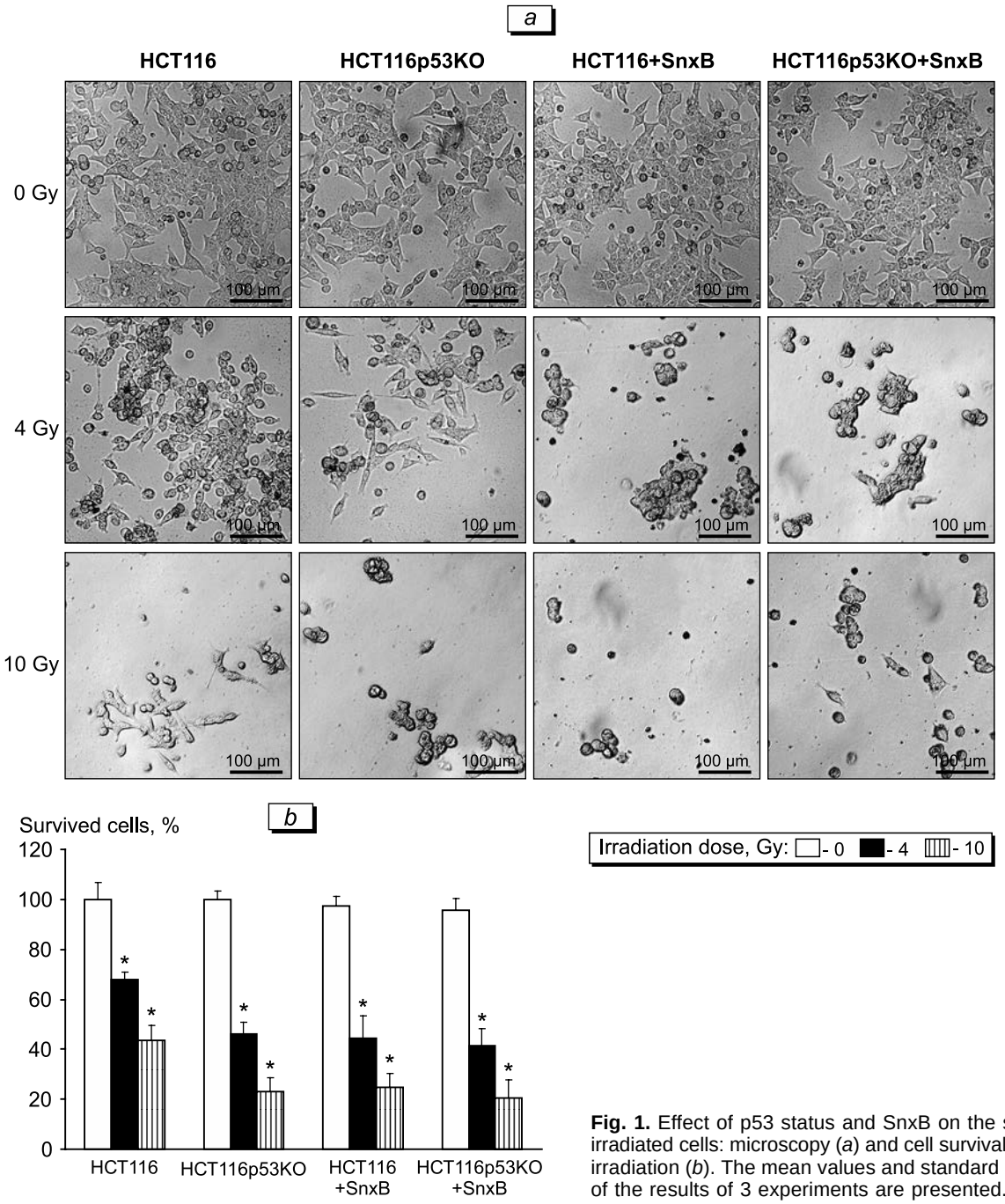

Fig. 1. Effect of p53 status and SnxB on the survival of irradiated cells: microscopy (*a*) and cell survival 72 h after irradiation (*b*). The mean values and standard deviations of the results of 3 experiments are presented.

in doses of 4 and 10 Gy, HCT116 cells were largely arrested in G_2/M, whereas the HCT116p53KO subline responded with apoptotic DNA fragmentation (hypodiploids, subG_1) (Fig. 2). After irradiation in a dose of 4 Gy, the proportion of G_2/M-phase HCT116 cells was 46%, while the subG1 population was relatively minor (23%). After irradiation in a dose of 10 Gy, the fraction of G_2/M-phase cells decreased to 36%, while the subG_1 fraction increased to 35%. In the presence of SnxB, the proportion of apoptotic HCT116 cells increased, whereas the percentage of G_2/M decreased. In contrast, SnxB had no effect on cell cycle distribution in the HCT116p53KO subline. Cell death depended on the dose: at 4 Gy 40% subG_1 and 17% G_2/M (no SnxB) *vs* 41 and 20%, respectively, with SnxB. At 10 Gy the phase distribution did not depend on SnxB.

Senescence marker and colony formation by irradiated cells with different p53 status. To investigate the influence of SnxB on radiation-induced senescence, HCT116 and HCT116p53KO cells were irradiated in the presence and absence of SnxB, incubated for 72 h, fixed, and stained for SA-β-Gal in a colorimetric

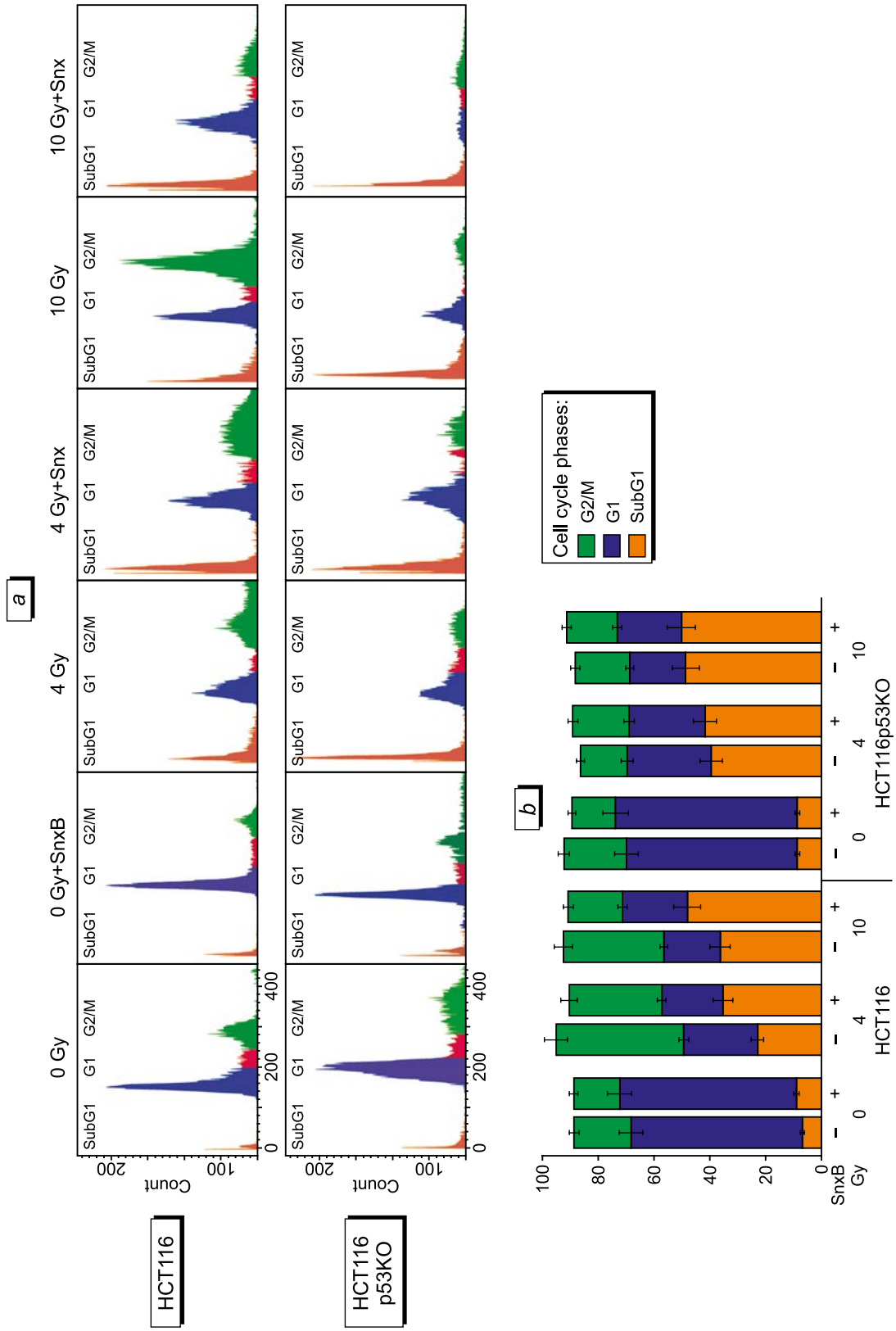

Fig. 2. Cell cycle distribution: histograms of the distribution of cell cycle phases (*a*) and fractions of cells in the corresponding phases (*b*). The mean values and standard deviations of the results of 3 experiments are presented.

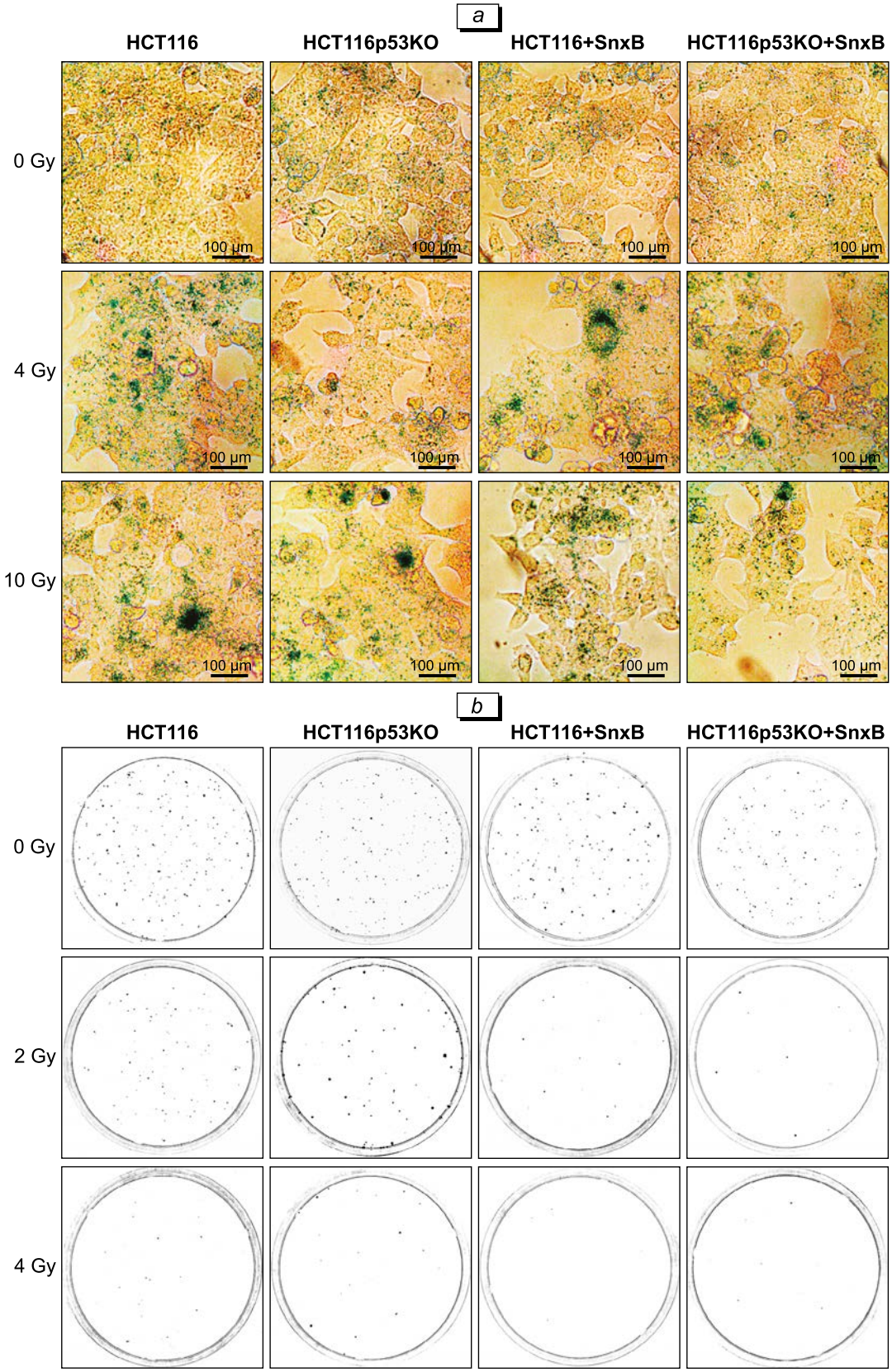

Fig. 3. Activity of SA-β-Gal and colony formation by HCT116 and HCT116p53KO cells irradiated with or without SnxB. Light microscopy (*a*) and number of colonies (*b*).

TABLE 1. SA-β-Gal Activity and Colony Formation of HCT116 and HCT116p53KO Cells after Irradiation in Different Doses and in the Presence of SnxB

Parameter	Irradiation dose, Gy	HCT116	HCT116p53KO	HCT116+SnxB	HCT116p53KO+SnxB
SA-β-Gal activity	0	4±1	4±1	2±1	3±1
	4	26±2	21±1	9±2*	15±3
	10	36±4	31±4	19±4*	32±2
Number of colonies	0	198±2	193±5	179±5	174±4
	4	86±9	55±8	21±4*	29±2
	10	23±5	13±2	6±2*	10±1

Note. *$p<0.05$ in comparison with the corresponding cells irradiated in the absence of SnxB (0 Gy).

reaction with the X-Gal substrate. In non-irradiated cultures or in cultured treated with SnxB alone, only solitary weakly stained SA-β-Gal$^+$ cells were seen (<5%). After irradiation in a dose of 4 Gy without SnxB, the fraction of senescent cells was ~27% in HCT116 cells and ~20% in the HCT116p53KO subline. After irradiation of HCT116 cell in the presence of SnxB, the percentage of SSA-β-Gal$^+$ cells decreased to ~9%. In the HCT116p53KO subline, the changes were statistically insignificant. After irradiation in a dose 10 Gy, the same tendency was revealed: SnxB attenuated the development of senescence in HCT116 cells, but not in HCT116p53KO subline (Fig. 3, *a*).

The results obtained in the analysis of mass cell populations demonstrate the role of transcription reprogramming in p53-dependent responses to ionizing radiation. It is necessary to find out whether SnxB affects cell survival and colony formation by single cells after irradiation. The mean number of colonies formed by non-irradiated HCT116 and HCT116p53KO cells was 180-200. After exposure to 2 Gy without SnxB, HCT116 cells formed more clones than HCT116p53KO subline. Irradiation in the presence of 10 μM SnxB significantly decreased the number of colonies in both lines ($p<0.05$). Irradiation in a dose of 4 Gy was sublethal; in both lines, the number of colonies was higher in the absence of SnxB (Fig. 3, *b*). Irradiation in a dose of 10 Gy was lethal for both cell lines. Thus, SnxB significantly decreased survival of HCT116 cells (a p53-dependent effect) and HCT116p53KO cells (a p53-independent effect).

Sensitization of tumor cells to the therapeutic irradiation by inhibiting CDK8/19 transcriptional protein kinases opens new vistas in studies of the mechanisms of cell responses to stress. Basically, the role of CDK8/19 in p53-dependent effects extends the list of cofactors of transcriptional reprogramming. From the practical viewpoint, CDK8/19 inhibition enhanced death of irradiated cells with wild-type and non-functional p53 depending on experimental conditions. These findings suggest that non-toxic selective blockers of transcriptional reprogramming can be a promising components of antitumor treatment regimens.

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