Targeting the survival kinase DYRK1B: A novel approach to overcome radiotherapy-related treatment resistance

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Abstract: BACKGROUND Cancer cell survival under stress conditions is a prerequisite for the development of treatment resistance. The survival kinase DYRK1B is a key regulator of stress survival pathways and might thereby also contribute to radiation resistance. Here we investigate the strategy of targeting DYRK1B in combination with ionizing radiation (IR) to enhance tumor cell killing under stress conditions. METHODS DYRK1B expression, ROS formation and DNA damage were investigated under serum-starvation (0.1% FBS), hypoxia (0.2%, 1% O_2) and IR. The combined treatment modality of IR and DYRK1B inhibition was investigated in 2D and in spheroids derived from the colorectal cancer cell line SW620, and in primary patient-derived colorectal carcinoma (CRC) organoids. RESULTS Expression of DYRK1B was upregulated under starvation and hypoxia, but not in response to IR. The small molecule DYRK1B inhibitor AZ191 and shRNA-mediated DYRK1B knockdown significantly reduced proliferative activity and clonogenicity of SW620 tumor cells alone and in combination with IR under serum-starved conditions, which correlated with increased ROS levels and DNA damage. Furthermore, AZ191 successfully targeted the hypoxic core of tumor spheroids while IR preferentially targeted normoxic cells in the rim of the spheroids. A combined treatment effect was also observed in CRC-organoids but not in healthy tissue-derived organoids. CONCLUSION Combined treatment with the DYRK1B inhibitor AZ191 and IR resulted in (supra-) additive tumor cell killing in colorectal tumor cell systems and in primary CRC organoids. Mechanistic investigations support the rational to target the stress-enhanced survival kinase DYRK1B in combination with irradiation to overcome hypoxia- and starvation-induced treatment resistances.

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Original Article

Targeting the survival kinase DYRK1B: A novel approach to overcome radiotherapy-related treatment resistance

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ABSTRACT

Background: Cancer cell survival under stress conditions is a prerequisite for the development of treatment resistance. The survival kinase DYRK1B is a key regulator of stress survival pathways and might thereby also contribute to radiation resistance. Here we investigate the strategy of targeting DYRK1B in combination with ionizing radiation (IR) to enhance tumor cell killing under stress conditions.

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Results: Expression of DYRK1B was upregulated under starvation and hypoxia, but not in response to IR. The small molecule DYRK1B inhibitor AZ191 and shRNA-mediated DYRK1B knockdown significantly reduced proliferative activity and clonogenicity of SW620 tumor cells alone and in combination with IR under serum-starved conditions, which correlated with increased ROS levels and DNA damage. Furthermore, AZ191 successfully targeted the hypoxic core of tumor spheroids while IR preferentially targeted normoxic cells in the rim of the spheroids. A combined treatment effect was also observed in CRC-organoids but not in healthy tissue-derived organoids.

Conclusion: Combined treatment with the DYRK1B inhibitor AZ191 and IR resulted in (supra-) additive tumor cell killing in colorectal tumor cell systems and in primary CRC organoids. Mechanistic investigations support the rational to target the stress-enhanced survival kinase DYRK1B in combination with irradiation to overcome hypoxia- and starvation-induced treatment resistances.

Introduction

Ionizing radiation (IR) primarily targets fast-dividing cells, leading to initial tumor regression, while non-dividing, quiescent tumor cells are more radioresistant and foster tumor recurrence, metastasis, and poor patient survival [1]. Stress conditions such as nutrient deprivation or hypoxia activate survival pathways to induce a quiescent state as defense mechanism and thereby acquire also treatment resistance [2]. Inhibiting key regulators within these pathways and exploiting these survival mechanisms give rise to new potential drug targets for combined treatment modalities with radiotherapy. The survival kinase DYRK1B (Dual-specificity tyrosine phosphorylation-regulated kinase 1B) has been identified as a pro-oncogenic kinase, which is upregulated in quiescent cancer cells enhancing survival and mediating treatment resistance [3,4]. Inhibition of DYRK1B in combination with chemotherapy has shown synergistic effects [5–9] but has not been investigated so far in combination with radiotherapy.

DYRK1B could contribute to radiation resistance on several levels. In response to serum-starvation, DYRK1B is upregulated in cell cycle-arrested cells as investigated in tumor cells derived from multiple tumor entities [6,8,10,11]. Hypoxia also promotes tumor cells to enter into a quiescent state [12], and DYRK1B was proposed as a potential therapeutic target to drive hypoxic cancer cells into a state of crisis [3,4,13]. DYRK1B also enhances cell survival via downstream upregulating gene expression of antioxidant proteins (e.g., ferroxidase and superoxide dismutases) thereby controlling ROS levels as observed in quiescent pancreatic tumor cells [10]. Furthermore, DYRK1B has been suggested as a mediator of transcription repression on damaged

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chromatin, orchestrating double-strand break (DSB) repair and chromosomal stability [14,15]. However, the effects of hypoxia on DYRK1B kinase activity are less consistent, e.g. Lee et al. demonstrated that oxygen-sensing, prolyl hydroxylase-mediated posttranslational modification of both DYRK1B and its paralogue DYRK1A is pivotal for their activation in glioblastoma cells [16,17].

Here, we investigate the regulation of DYRK1B as potential survival mechanism in colorectal carcinoma (CRC) cells in 2D and 3D cellular systems and in patient-derived CRC organoids under serum-starvation, hypoxia, and in response to irradiation and probe the combined treatment modality of IR with a specific DYRK1B kinase inhibitor.

Materials and methods

Cell lines, 3D spheroids and patient-derived organoids

The human colorectal carcinoma cell line SW620 (a gift of Janine T. Erler, University of Copenhagen) was cultured in DMEM (Gibco; 41966–029) supplemented with 10 % (v/v) fetal bovine serum (Gibco; 10270–106); 1 % (v/v) penicillin–streptomycin (Gibco; 15140122), and 1 % (v/v) GlutaMAX (Gibco; 35050–61). All cells were tested every 3–4 months for mycoplasma contamination (MycoAlert, Lonza). Cells were cultured in a 5 % CO_2 humified atmosphere at 37 °C. For hypoxic conditions cells were kept in a 0.2 % or 1 % O_2, 5 % CO_2 incubator (*In vivo*$_2$ 300-Ruskinn; Hypoxia Incubator, Siemens) at 37 °C. SW620-derived spheroids were formed by seeding 5000 cells/well into Nucleon™ Sphera™ 96-well plates, with all details on treatment and imaging given in the Supplementary document. CRC organoids were generated from tumor liver metastases (a gift from Chantal Pauli, University Hospital Zurich) and normal tissue organoids derived from healthy colorectal tissue (a gift from Michael Scharl, University Hospital Zurich). See culturing conditions in the Supplementary document.

Generation of genetically modified cells

A pLKO.1 puro lentiviral backbone (Addgene plasmid #21915), was used for constructing doxycycline inducible SW620 shDYRK1B cell lines [18]. Sequence data are given in the Supplementary document. The shDYRK1B construct was induced with doxycycline (1 μg/mL, Sigma; D9891) 72 h prior to starting the experiment. For the hypoxia-sensing SW620_HBR-6U cell line, transfection and transduction was caried out with HBR-6U plasmid (Addgene plasmid #42621) as described and verified in the Supplementary document [19]. After transduction, cells were cultured in hypoxic conditions (1 % O_2) and selected by fluorescence-activated cell sorting (FACSAria™ III Cell Sorter, BD Bioscience).

Cell treatment and irradiation

AZ191 was dissolved in DMSO and diluted in 10 % or 0.1 % FBS containing medium. Irradiation was performed at 6.63 Gy/minute (RS-2000 225 kV irradiator, Rad Source) 3 h after AZ191 treatment. The proliferative activity of SW620 cells was determined with the AlamarBlue assay (Invitrogen; DAL1100) [20]. For shDYRK1B knockdown, cells were pretreated in normal medium -/+ dox (1 μg/mL) for 72 h. The growth medium was then changed to 10 % or 0.1 % FBS -/+ dox (1 μg/mL) and irradiated 3 h thereafter. Clonogenic cell survival was performed as previously described [21]. For ROS scavenging experiments, cells were treated with N-acetylcysteine (NAC; 1 mM or 5 mM) 30 min after IR. Detailed protocols for western blotting, RT-qPCR, and cell cycle analysis and quantification are given in the Supplementary document.

ROS generation assay

ROS measurements were performed 20 h after treatment start using CM-H_2DCFDA (5 μM, Invitrogen; C6827) for total ROS measurements and MitoSox Red (5 μM, Invitrogen; M36008) and MitoPY1 (10 μM Tocris; 4428) for mitochondrial ROS measurements. Cells were incubated for 30 min at 37 °C with the agents prior to resuspension in ice-cold PBS and immediate analysis by flow cytometry.

γH2AX staining, quantification and immunofluorescence

γH2AX foci were stained in PFA-fixed and methanol-permeabilized cells with the rabbit anti-phospho-Histone H2A.X (Ser139) antibody (Cell Signaling Technology; #9718, 1:500) followed by the goat anti-rabbit Alexa Fluor 488 secondary antibody (Invitrogen; #A11070, 1:500) and analyzed by flow cytometry. Cells with pan-nuclear γH2AX staining were excluded from the analysis using the gating strategy outlined in [22]. For imaging γH2AX foci, cells were seeded on cover glasses and antibody-stained as described above including DNA staining with Hoechst 33342 (MedChemExpress; HY-15559A, 5 μg/mL) and analyzed by fluorescence microscopy.

Statistical analysis

The data were analyzed with GraphPad Prism v9.5.1. using two-tailed unpaired *t*-test and ANOVA with Dunnett's or Tukey's correction for multiple comparisons. For all experiments: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$.

Results

Serum starvation has previously been determined to upregulate DYRK1B expression in tumor cells derived from different tumor entities thereby mediating cell cycle arrest as a stress survival mechanism [8,11] (Supplementary Fig. 1). Here, we examined the expression level of DYRK1B after serum-starvation (0.1 % FBS) by RT-qPCR and western blotting in SW620 colorectal carcinoma cells. Furthermore, DYRK1B expression was also probed following incubation under hypoxic conditions (0.2 % and 1 % O_2) and irradiation as additional stress factors (Fig. 1B, C). Both serum-starvation and hypoxia significantly increased DYRK1B expression over time on the mRNA and protein level (Fig. 1A, B). Irradiation did not enhance DYRK1B basal expression levels (Fig. 1C). Increased DYRK1B kinase activity could also be identified in response to serum-starvation and hypoxia by an increase in the phosphorylation status of the DYRK1B-substrate and cell cycle inhibitor p27Kip1 [23,24].

To investigate the relevance of DYRK1B as a stress regulator in combination with irradiation, tumor cells were irradiated under normal and serum-starved conditions with and without the small molecule DYRK1B inhibitor AZ191 [5,25]. Cellular incubation with AZ191 decreased phosphorylation of the DYRK1B substrate pSer10p27 in serum-starved SW620 cells (Fig. 2A). Under 10 % FBS conditions, AZ191 (1 and 3 μM) only induced a minor antiproliferative effect, which was further enhanced when combined with 2 Gy of IR. Interestingly, significantly reduced proliferation of SW620 cells was already induced by AZ191 when incubated under serum-starved condition alone, which was further decreased when combined with 2 Gy of IR (Fig. 2B). Likewise, clonogenic survival in response to irradiation was reduced by the DYRK1B-inhibitor when tumor cells were preincubated with AZ191 and irradiated under serum-starved conditions. Combined treatment of AZ191 and increasing doses of IR resulted in a strong supra-additive effect under serum-starved conditions but not under 10 % FBS conditions (Dose Modifying Factor at Survival Fraction 0.5; $DMF_{SF\ 0.5} = 1.51$ vs $DMF_{SF\ 0.5} = 1.10$ respectively) (Fig. 2C). Control experiments were performed with SW620 cells, stably transduced with a doxycycline-inducible scrambled shRNA (shCTR) vector or an inducible DYRK1B-directed shRNA (shDYRK1B) expression vector (Fig. 2D). In line with the data obtained with the small molecule DYRK1B-inhibitor, a strong antiproliferative effect on DYRK1B knockdown was only observed in cells growing under starved conditions (Fig. 2E), which was further

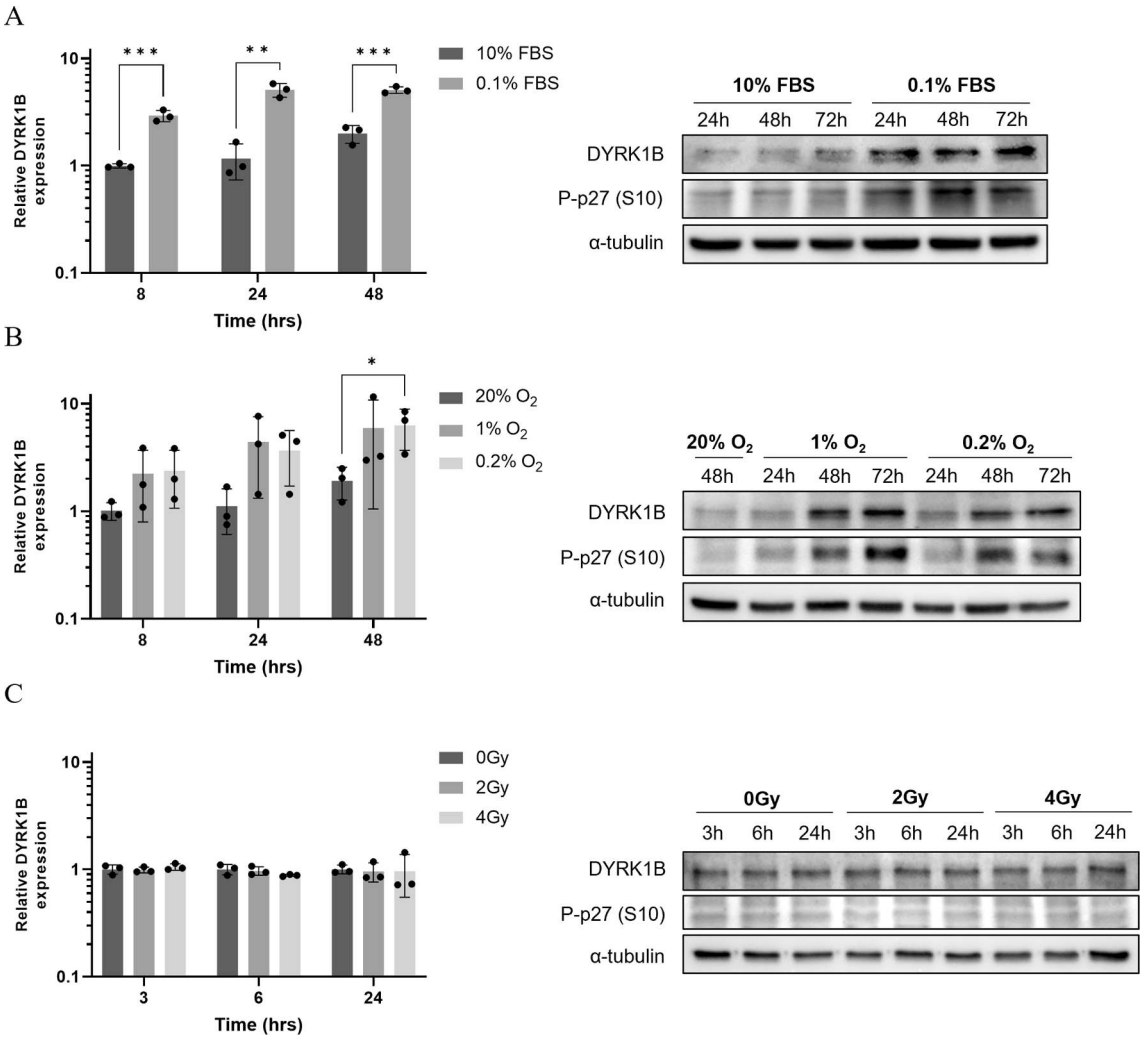

Fig. 1. Upregulation of DYRK1B under stress conditions in SW620 colorectal carcinoma cells. Upregulation of DYRK1B determined by RT-qPCR (left) and western blotting, including its substrate phospho-p27 (Ser10), (right) in normal conditions (10 % FBS, 20 % O_2, 0 Gy) compared to serum-starvation (0.1 % FBS) **(A)**, hypoxia (1 %, 0.2 % O_2) **(B)**, and ionizing radiation (IR;2Gy, 4 Gy) **(C)**. Bar graphs represent the mean ± SD of three independent experiments.

enhanced in combination with irradiation (Fig. 2F).

DYRK1B controls the expression of ROS scavenger proteins and thereby contributes to cellular survival under stress conditions [6,10]. Enhanced total ROS levels were determined when SW620 cells were incubated with increasing concentrations of AZ191 alone and in combination with IR (Fig. 3A, Supplementary Fig. 4). Mitochondrial superoxide, detectable by mitoSOX, was most susceptible to DYRK1B inhibition with a 1.5-fold increase in comparison to untreated cells (Fig. 3B). Interestingly, we also detected increased expression of SOD3 and HEPH under starvation conditions, while SOD1 and SOD2 expression levels were not affected by starvation. Of note, DYRK1B downregulation in doxycycline inducible SW620shDYRK1B cells resulted in reduced expression of both SOD3 and HEPH suggesting a mechanistic link between DYRK1B inhibition, HEPH and SOD3 expression and deregulated ROS levels (Supplementary Fig. 2).

Next, we determined DNA double strand breaks 24 h after treatment with AZ191 and IR alone and in combination, which might result from increasing ROS levels generated over time or by interference of DYRK1B in DNA double strand break repair [14]. An increased level of γH2AX foci was observed in the nuclei of SW620 cells following treatment with AZ191 alone, which was further enhanced when combined with 2 Gy of IR (Fig. 3D). To determine DNA damage due to DYRK1B-deregulated ROS formation, cells were treated with N-acetylcysteine (NAC) 30 min after IR to avoid scavenging ROS produced by IR-mediated radiolysis of water and to more specifically address ROS enhancement due to DYRK1B inhibition. (Supplementary Fig. 3). Interestingly NAC efficiently scavenged ROS formed in AZ191-pretreated, unirradiated and irradiated cells, and also abrogated formation of yH2AX foci in unirradiated cells. In irradiated cells, NAC (added following irradiation) only partially reduced formation of yH2AX foci, suggesting additive DNA damaging by DYRK1B inhibition and irradiation.

DYRK1B mediates cell cycle arrest under serum-starvation and thereby acts as a negative regulator of G0/G1-S phase transition in quiescent tumor cells [10,23,25,26]. To corroborate this role of DYRK1B, SW620 cells were treated with increasing concentrations of AZ191, which resulted in a gradual decrease in the amount of serum-starved cells residing in the G0/G1 cell cycle phase and accumulated into the radiosensitive G2 cell cycle phase. This DYRK1B-dependent effect was less pronounced in tumor cells growing under 10 % FBS conditions (Fig. 3E).

To investigate DYRK1B-inhibition alone and in combination with irradiation under normoxic and hypoxic conditions, we opted for a physiologically more relevant 3D-spheroid model. SW620 cells were transduced with a hypoxia-sensing vector (HBR-6U) to identify hypoxia in the core of the spheroids (Fig. 4B and Supplementary Fig. 5). Spheroids were treated with the DYRK1B inhibitor and irradiation alone and in combination, and microscopically analyzed three days thereafter (Fig. 4A and B). Interestingly, spheroid treatment with AZ191 alone

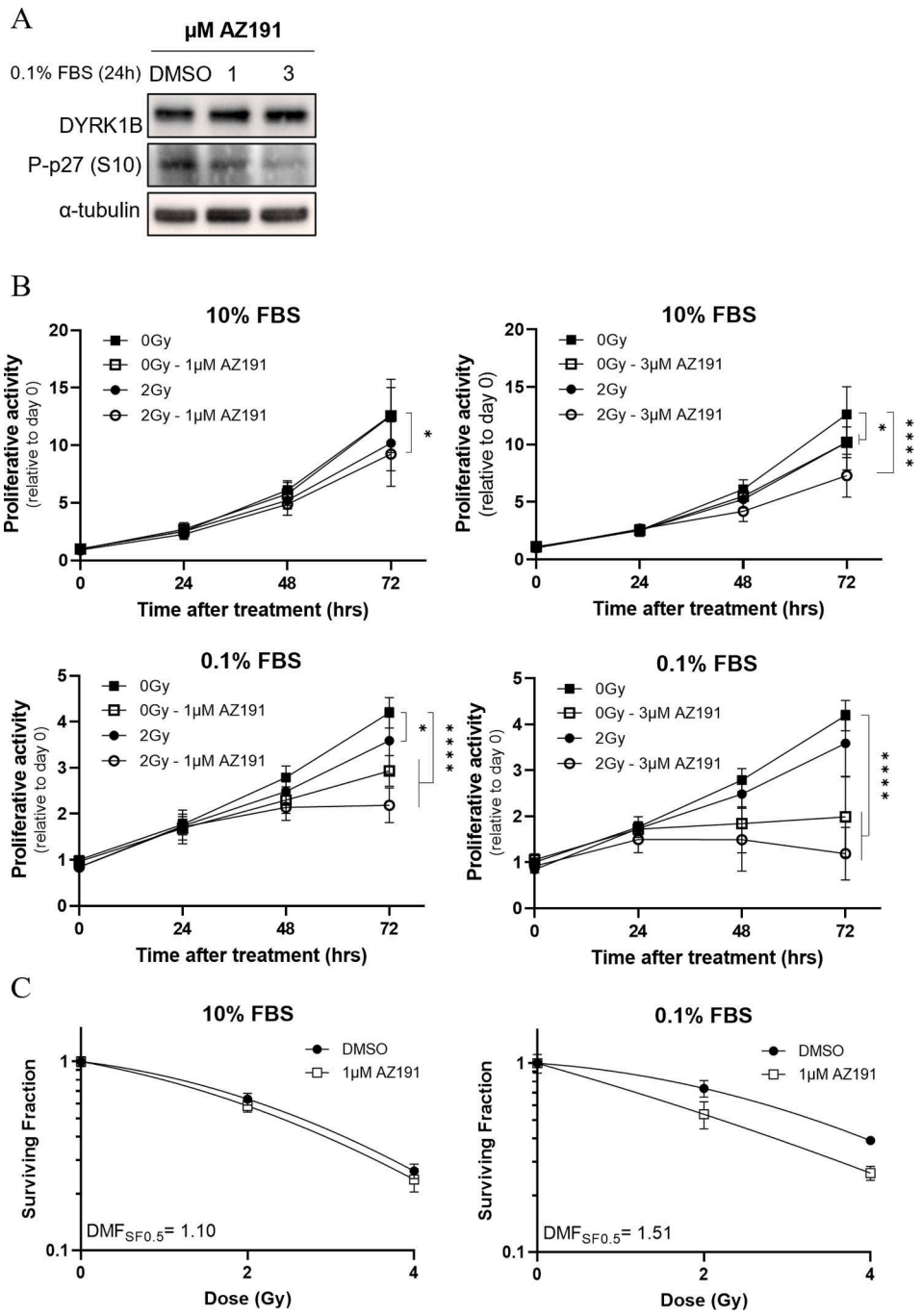

Fig. 2. Differential cellular response to DYRK1B-inhibition and irradiation under normal and serum-starved conditions. (A) DYRK1B inhibition with AZ191 (1 and 3 μM for 24 h in 0.1 % FBS) downregulates the phosphorylation status of p27. **(B)** SW620 cells were treated with increasing concentrations of AZ191(1 and 3 μM) alone and in combination with IR (2 Gy) in normal (10 % FBS) and serum-starved (0.1 % FBS) conditions. Cells were irradiated 3 h after AZ191 treatment. The proliferative activity was determined over 72 h by Alamar Blue assay. **(C).** Clonogenic cell survival of SW620 cells treated with 1 μM AZ191 and 2 or 4 Gy of IR in either 10 % or 0.1 % FBS containing media. 24 h after IR, single cells were replated into normal conditions (10 % FBS), and colonies were fixed, stained and counted 14 days thereafter. **(D)** Western blotting of cellular lysates following expression of two doxycycline-inducible shRNA constructs for 72 h directed against DYRK1B (sh1B#1, #2; or scrambled control, shCTR) in 10 % and 0.1 % FBS, cells expressing construct sh1B#2 were used for further experiments. **(E, F)** Proliferative activity of SW620 cells following DYRK1B knockdown over 72 h in 10 % FBS or 0.1 % FBS alone (E) and combination with 2 Gy of IR (F). DMF$_{SF0.5}$, dose modifying factor at 50 % survival. Results represent the mean ± SD of three independent experiments. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

caused cell death (propidium iodine positivity) specifically in the core of the spheroid, while irradiation induced cell death throughout the spheroid, also targeting the outer rim of the spheroid (Fig. 4 C, D). Combining AZ191 with IR induced a dual effect targeting both the inner hypoxic core as well as rim cells of the spheroid, resulting in supra-additive cell killing (Fig. 4E and Supplementary Table 1).

Eventually we probed our findings in clinically more relevant primary patient-derived colorectal carcinoma organoids. Interestingly, a significant decrease in organoid integrity was observed in these colorectal tumor-derived organoids in response to treatment with AZ191 and

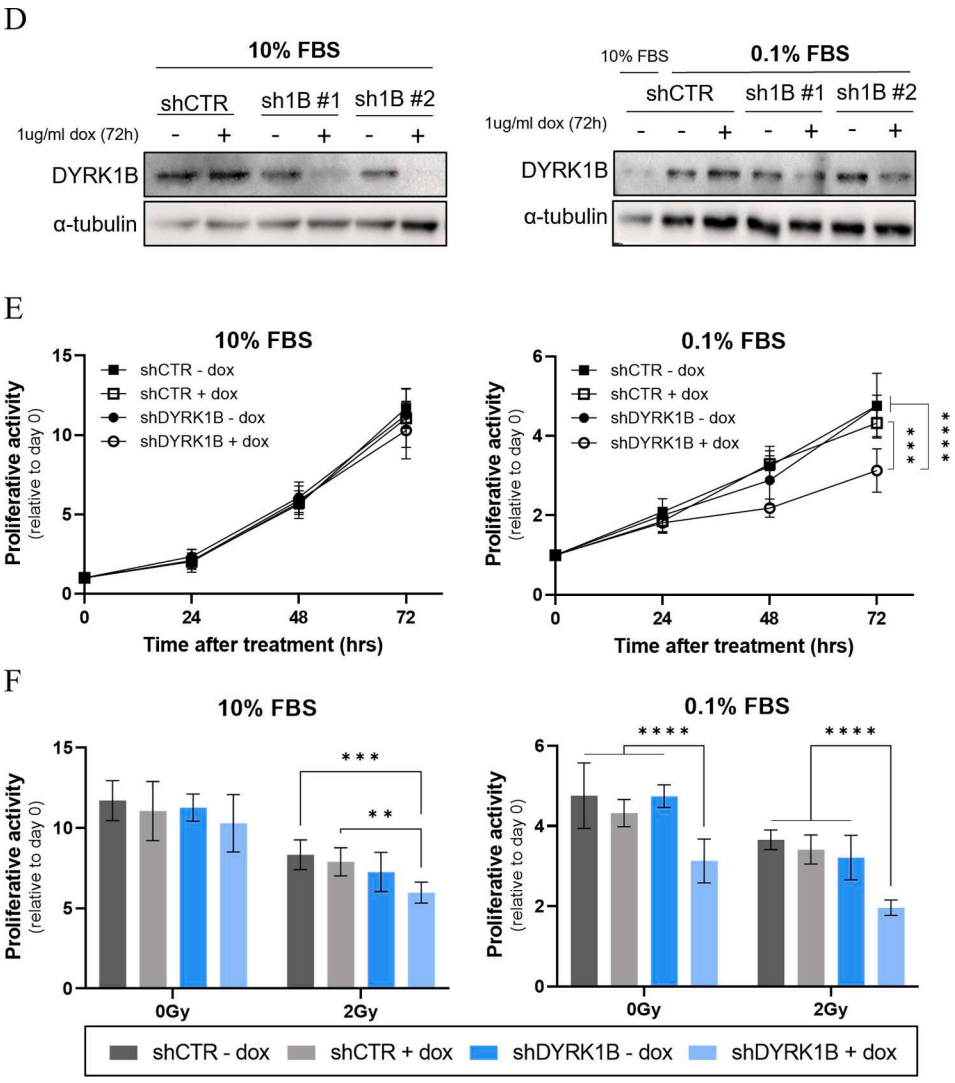

Fig. 2. (*continued*).

IR alone and in combination (Fig. 5A and Supplementary Table 1) corroborating our data in SW620 cells. DYRK1B in these organoids was also significantly upregulated under serum-starvation (0.1 % FBS) and hypoxia (0.2 % O_2), and only partially in response to IR (Fig. 5C). In contrast, only minimal loss of organoid integrity was observed when treating normal-tissue patient-derived colorectal organoids (Fig. 5B).

Discussion

Several strategies have been developed during the last decades to overcome the hurdle of tumor hypoxia as part of combined treatment modalities with radiotherapy [27–36]. Hypoxic radiosensitizers, hypoxia-activated prodrugs and molecular-targeting agents (e.g., HIF-inhibitors) are preferentially effective in hypoxic parts of the tumor. Other approaches (e.g., tumor vasculature normalization [37]) aim to increase oxygen availability in the tumor and subsequently to reduce tumor hypoxia and radiation resistance.

The combination of radiotherapy with inhibitors targeting resistance mechanisms that are upregulated under stress conditions represents a novel strategy to overcome hypoxia-induced radioresistance. This strategy corresponds to the concept of biological cooperation. While ionizing radiation kills primarily well-oxygenated cells, such inhibitors of survival processes will prevent stress-induced cell cycle arrest, and subsequently drive quiescent tumor cells under hypoxia and/or nutrient limited conditions either directly into cell death or into re-entry of a proliferative and thereby more IR-sensitive state.

DYRK1B is a dual-specific kinase known to be upregulated under nutrient deprivation. While regulation of DYRK1B under hypoxia has not been well investigated so far, we demonstrated in established colorectal tumor cells and in primary colorectal carcinoma organoids, that hypoxia increases DYRK1B mRNA- and protein levels. However, more detailed investigations are required to fully understand its regulation under hypoxia, as for example Lee et al. also demonstrated that hydroxylation by the oxygen-sensing prolyl hydroxylase PHD1 is pivotal for the activation of DYRK1B and DYRK1A in glioblastoma cells [16,17].

DYRK1B inhibition with a small molecule inhibitor and shRNA-based knockdown of DYRK1B interferes with multiple processes relevant for radiotherapy treatment resistance. We showed that DYRK1B inhibition deregulates stress-related cell cycle arrest and that combined treatment with IR results in increased ROS formation and DNA damage. DYRK1B counteracts G0/G1 – S phase transition via different mechanisms [3,8]. One of DYRK1B's downstream targets is stabilizing the CDK inhibitor p27 by its phosphorylation on serine 10 (S10), thereby preventing cell cycle progression [23]. Our results show that DYRK1B overexpression and subsequent phosphorylation of p27(S10) is induced by serum-starvation and hypoxia, but not by IR. At this stage we could not identify, which of these processes are most relevant for enhanced tumor cell killing by the combined treatment modality. More important though, we

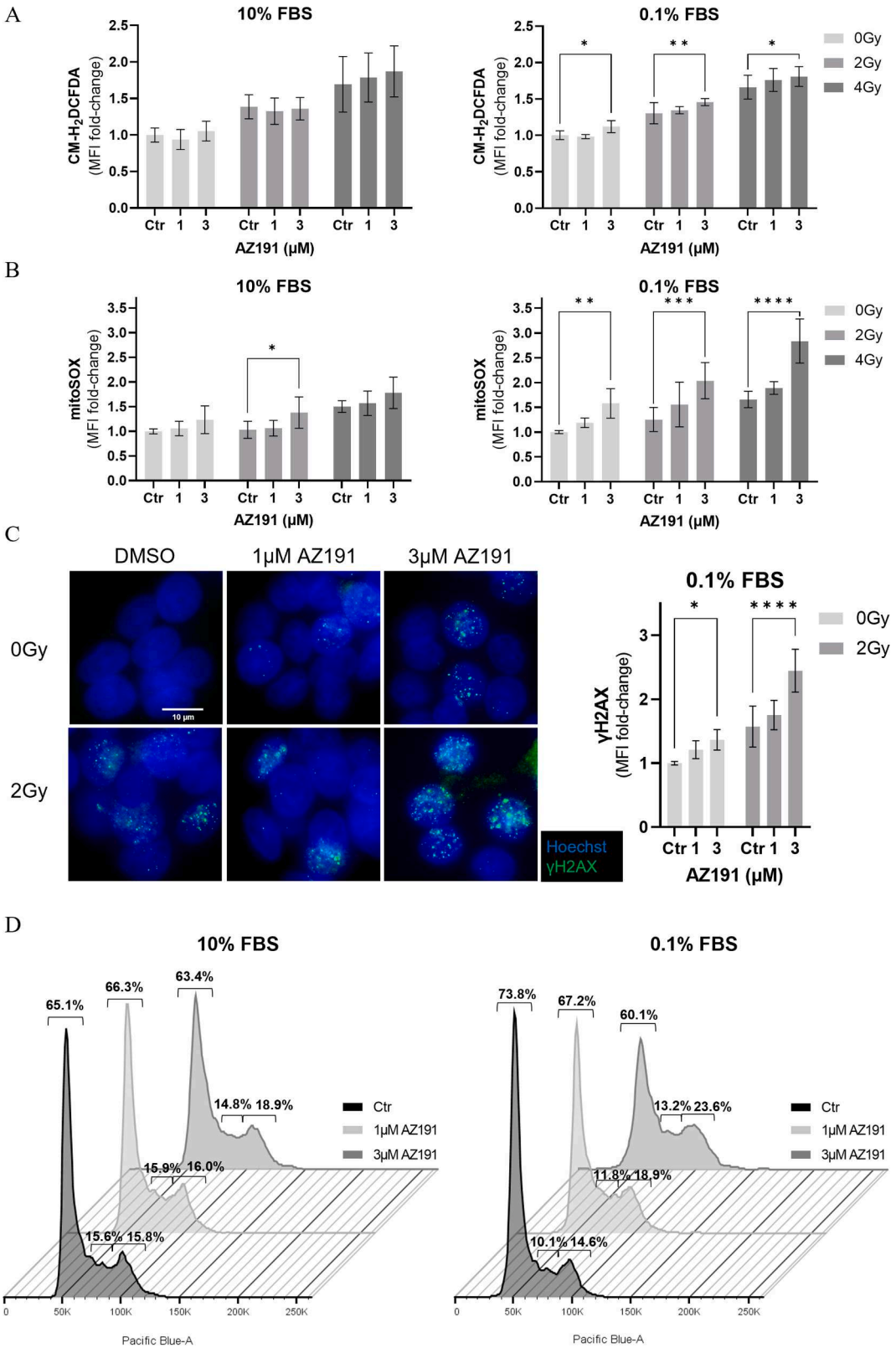

Fig. 3. Cellular ROS levels, DNA damage and cell cycle distribution under normal and serum-starved conditions in response to treatment with AZ191 and IR alone and in combination. Total ROS levels (**A**, CM-H_2DCFDA) and mitochondrial superoxide levels (**B**, mitoSOX) were determined by flow cytometry 20 h after treatment start with AZ191 and IR (alone and in combination) in 10 % FBS or 0.1 % FBS conditions. Cells were irradiated 3 h after AZ191 treatment. **(C)** Residual γH2AX foci 24 h after treatment with AZ191 and 21 h following 2 Gy of IR in serum-starved conditions (0.1 % FBS), imaged with fluorescent microscopy (left) and quantified by flow cytometry (right). **(D)** Cell cycle analysis 48 h after AZ191 treatment in 10 % and 0.1 % FBS by Hoechst 33342 and anti-phospho-Histone H3 staining and flow cytometry. MFI, mean fluorescent intensity. Bar graphs represent the mean ± SD of three independent experiments.

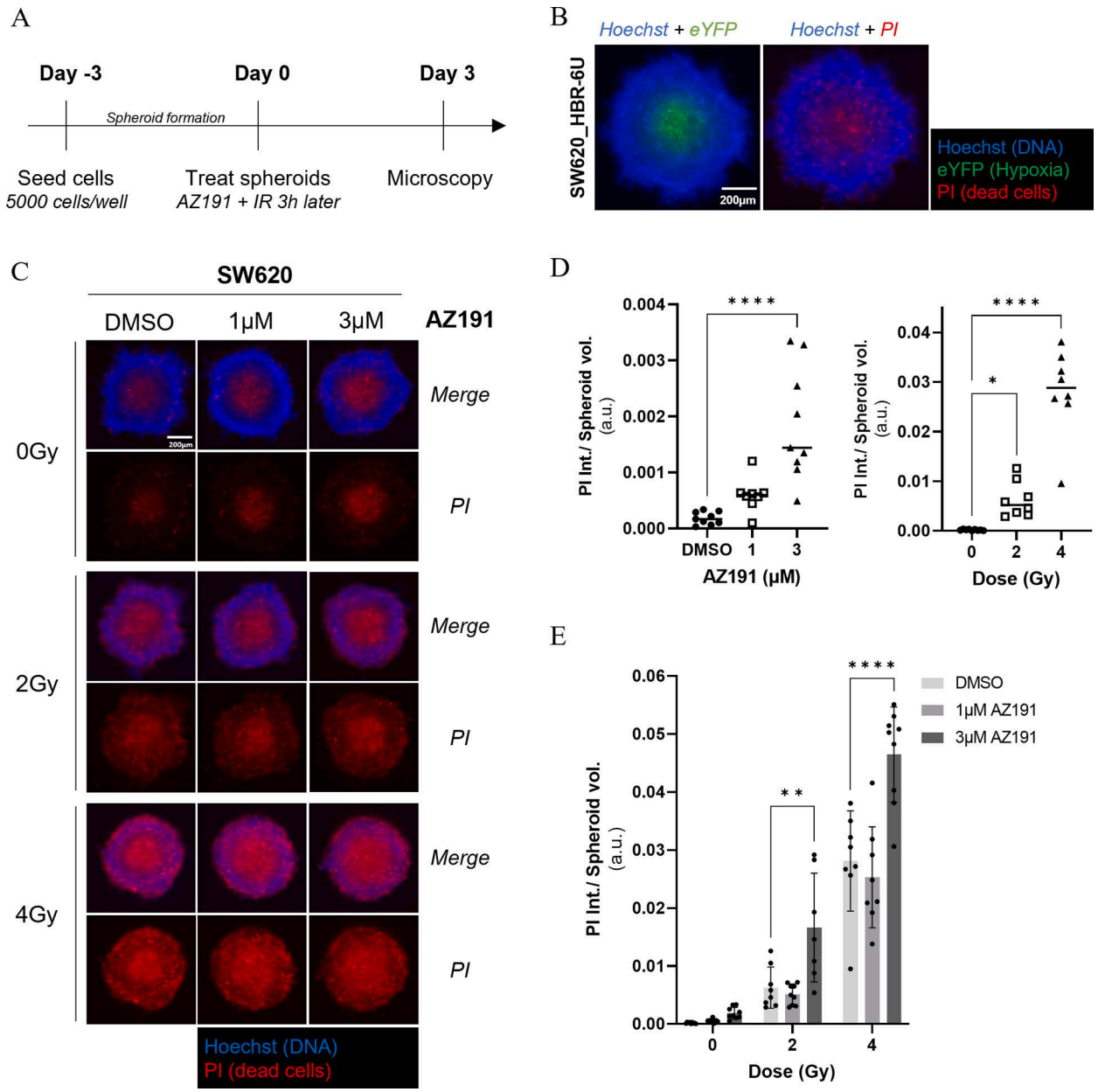

Fig. 4. Differential cell killing in SW620-derived tumor spheroids with a hypoxic core in response to AZ191 and IR. (A) Design of the experiment: spheroids were formed for 3 days, treated with AZ191 and IR (3 h thereafter) and analyzed by fluorescence live-cell imaging 3 days following treatment start. **(B)** SW620-derived spheroid transduced with the HBR-6U hypoxia-sensing construct depict a hypoxic core containing a limited portion of propidium iodide (PI)-stained necrotic cells. **(C)** 3D SW620-derived spheroids treated with increasing concentrations of AZ191 (1, 3 µM) and IR (0, 2, 4 Gy). Spheroids were stained with Hoechst 33,342 (DNA) and PI (dead cells). **(D)** Quantification of the fluorescent intensity of PI over the total spheroid volume (PI int./spheroid vol.) after treatment with AZ191 (left) and IR (right) alone, and in combination **(E)**. Dot plots and bar graphs represent the mean ± SD of three independent experiments.

identified that the DYRK1B kinase inhibitor successfully targeted the hypoxic core of the tumor spheroid while irradiation preferentially targeted the normoxic rim of the spheriod resulting in spatial cooperation-based supra-additive tumor cell killing. Using fluorescence imaging, normoxic and hypoxic, viable and dead tumor cells could be semi-quantitatively imaged in treated tumor spheroids with high spatial resolution. Interestingly, we could also demonstrate a combined treatment response in primary tumor organoids but not in organoids derived from healthy tissue, which indicates a potential therapeutic window and the relevance of DYRK1B-controlled processes in tumor tissue. This differential response also correlates with other results observing higher expressed levels of DYRK1B in colorectal carcinoma tissue than in paired normal colorectal tissue [38]. Subsequent studies have consistently demonstrated that DYRK1B is expressed in few normal tissues (e.g. testis and skeletal muscle), but is overexpressed in various cancer types (e.g. colorectal, lung, breast, ovarian, pancreatic carcinoma) in which it exhibits prosurvival and protumorogenic roles (for a comprehensive overview, the reader is referred to [3,4].

In summary, we demonstrated that not only serum-starvation but also hypoxia promotes DYRK1B overexpression. Inhibition of DYRK1B in quiescent tumor cells could thereby drive tumor cells on its own into crisis or into a more radiosensitive cell cycle phase. This novel strategy was investigated in 2D and 3D cellular colorectal tumor systems with highly promising results also in primary tumor organoids without showing toxicity in normal tissue organoids. Detailed mechanistic- and efficacy-oriented studies in additional tumor models alone and in combination with irradiation are now required to further corroborate the potency of this novel strategy to overcome the hurdle of tumor hypoxia.

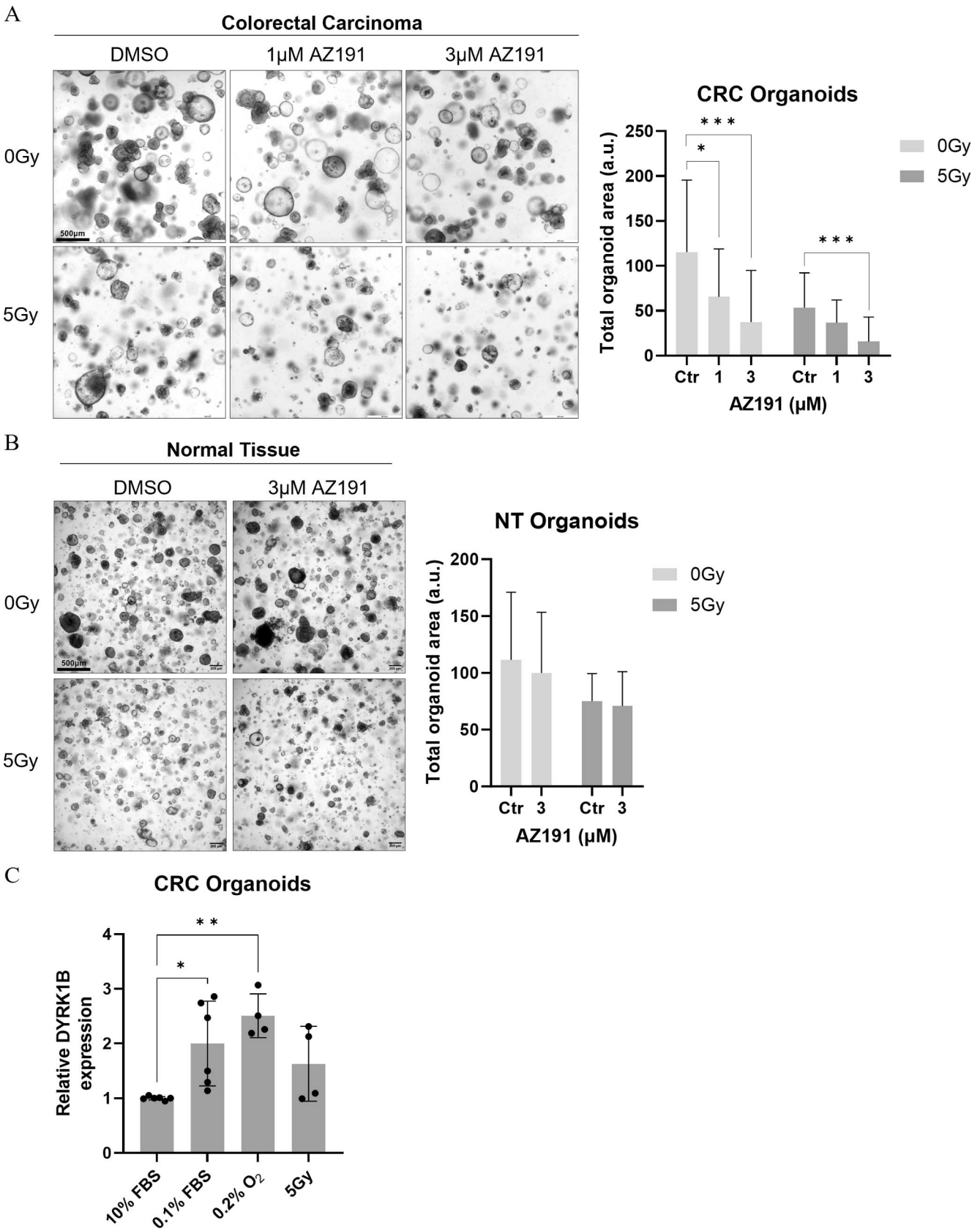

Fig. 5. Integrity of colorectal carcinoma and normal tissue colorectal patient-derived organoids following treatment with AZ191 and IR. (A) Images and area quantification of CRC organoids seven days after plating and treatment with AZ191 and IR (5 Gy). **(B)** Images and area quantification of normal tissue (NT) colorectal organoids treated with AZ191 and IR. **(C)** DYRK1B expression in CRC organoids determined 24 h following starvation (0.1 % FBS), hypoxia incubation (0.2 % O_2) and IR (5 Gy) with RT-qPCR. CRC, colorectal carcinoma. Bar graphs represent the mean ± SD of three (A, C) and two (B) independent experiments.

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CRediT authorship contribution statement

Claire Beckers: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Visualization, Writing – original draft, Writing – review & editing. **Lazaros Vasilikos:** Conceptualization, Methodology, Formal analysis, Investigation. **Alba Sanchez Fernandez:** Investigation. **Lorena Moor:** Investigation. **Martin Pruschy:** Conceptualization, Methodology, Resources, Funding acquisition, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.radonc.2023.110039.

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