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ORIGINAL MANUSCRIPT

Knockdown of hnRNPK leads to increased DNA damage after irradiation and reduces survival of tumor cells

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Abstract

Radiotherapy is an important treatment option in the therapy of multiple tumor entities among them head and neck squamous cell carcinoma (HNSCC). However, the success of radiotherapy is limited by the development of radiation resistances. Heterogeneous nuclear ribonucleoprotein K (hnRNPK) is a cofactor of p53 and represents a potential target for radio sensitization of tumor cells. In this study, we analyzed the impact of hnRNPK on the DNA damage response after gamma irradiation. By yH2AX foci analysis, we found that hnRNPK knockdown increases DNA damage levels in irradiated cells. Tumor cells bearing a p53 mutation showed increased damage levels and delayed repair. Knockdown of hnRNPK applied simultaneously with irradiation reduced colony-forming ability and survival of tumor cells. Taken together, our data shows that hnRNPK is a relevant modifier of DNA damage repair and tumor cell survival. We therefore recommend further studies to evaluate the potential of hnRNPK as a drug target for improvement of radiotherapy success.

Introduction

Tumors of epithelial origin are responsible for more than 80% of all cancer-related deaths in the Western world (1). Radiotherapy is an important treatment option for many tumors, especially squamous cell carcinoma of the head and neck (HNSCC), where radiotherapy is indicated alone, as radio chemotherapy or in an adjuvant setting in the broad majority of all patients (2,3). Unfortunately, the development of radiation resistances limits the success of radiotherapy.

Heterogeneous nuclear ribonucleoprotein K (hnRNPK) is overexpressed in HNSCC (4–6) and regulated upon irradiation (7). HnRNPK interacts directly with RNA and DNA and is involved in the regulation of gene expression at different levels (8). HnRNPK predominantly localizes to the nucleus but its ability to shuttle from the nucleus to the cytoplasm is one of its key regulatory features.

In different tumor entities cytoplasmic accumulation of hnRNPK could be shown (9,10) and is associated with poor prognosis in colorectal, prostate, nasopharyngeal cancer and hepatocellular and oral squamous cell carcinoma (4–6,11–14). Additionally the cytoplasmic accumulation of hnRNPK could be related to increased cell migration and thus potentially increased metastasis (15). Therefore, an involvement of hnRNPK in tumorigenesis is discussed.

Recently, hnRNPK has been identified as a cofactor of p53, which is a key factor determining the fate of cells after they had suffered DNA damage (8,16). Under normal conditions, the half-life of p53 is short and the protein level is kept low, but cellular stress leads to p53 stabilization and increase in DNA binding capability. As a result, p53 responsive genes are transcribed and their gene products determine the fate of the cell (cell cycle arrest, repair, apoptosis) (17). HnRNPK acts as a cofactor of p53. It could be shown that phosphorylation of hnRNPK and p53 through ataxia telangiectasia mutated (ATM) and ataxia telangiectasia and Rad3-related protein (ATR) initiate the DNA

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Abbreviations

DDR	DNA damage response
DSB	double strand break
GOF	gain-of-function
Gy	Gray
hnRNP K	heterogeneous nuclear ribonucleoprotein K;
HNSCC	head and neck squamous cell carcinoma
pCHK1/2	phosphorylated check-point kinase 1/2
SSB	single strand break

damage response (DDR) (16). Both proteins together are able to bind to p53 promoter regions, which leads to the transcription of DDR genes, such as p21. Without DNA damage, hnRNPK and p53 are ubiquinated by HDM2 and thus marked for proteasomal degradation (8). Mutation of the p53 gene is the most frequent gen-specific alternation in tumor cells, also in HNSCC (18), which makes the interaction between hnRNPK and mutant p53 particularly interesting.

To sum it up, hnRNPK is part of the DDR and dysregulated in many tumors. Due to the challenging problem of radiation resistances in head and neck tumors, we were wondering about the suitability of hnRNPK as a potential drug target in cancer therapy. Therefore, our goal was to gain a better understanding of the role of hnRNPK in the DDR, especially in the light of mutated p53.

To take into account the interaction of hnRNPK and p53, we choose two epithelial tumor cell lines with different p53 status for our analysis. With the help of a yH2AX foci analysis, we quantified DNA damage and its dynamic after irradiation. With a colony-forming assay, we determined the surviving fraction after irradiation dependent on the hnRNPK level. Finally, western blot analysis was used to shed light on DDR signaling.

Materials and methods

Tumor cell lines and cell culture

The non-small cell lung cancer (NSCLC) cell line A549 was purchased from DSMZ (German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany) in 2011. The head and neck squamous cell carcinoma (HNSCC) cell line HNSCCUM-02T was previously established and characterized in our laboratory (19). The identity of both cell lines was verified by STR analysis in 2014 by the DSMZ. All cells used in the experiments directly originate from the stocks, which were verified. Cells were maintained in DMEM/Ham's F12 (Sigma-Aldrich, St. Louis, MO) supplemented with 5% FCS (fetal calf serum; Sigma-Aldrich, St. Louis, MO) and antibiotics (100 U/ml penicillin and 100 mg/ml streptomycin) at 37°C in 5% CO_2.

HnRNPK knockdown and gamma-irradiation

SiRNA transfection was performed according to the manufacturer's protocol using either hnRNPK siRNA (Silencer® Select siRNA, s6738, predesigned, Ambion Applied Biosystems, Darmstadt, Germany) or positive and negative controls (Silencer® Select siRNA GAPDH and negative control #2).

yH2AX assay

The yH2AX immunofluorescence assay is a sensitive method for the quantification of DNA double strand breaks (DSB) (20) and their disappearance a measure of the efficiency of DNA repair (21). According to published data, showing that phosphorylation of γH2AX is a fast reaction upon DNA damage, we wanted to cover the early time points and chose short time intervals. For the assay cells were harvested 30 min, 1, 1.5, 2, 4 and 48 h post-irradiation as previously described (22). Each sample was analyzed with at least 50 cells. Statistical analysis of foci count data was performed by fitting a linear mixed model to the log (foci per cell) with treatment, radiation dose and time as covariates. Interactions were included in the model. These analyses were done by using PROC MIXED from SAS 9.4.

Expression analysis by SDS-PAGE and western blotting

For expression analyses, cells were harvested 15 min, 1 and 4 h after irradiation. 20–30 µg of total protein was loaded onto 10–12% acrylamide gels and subjected to SDS-PAGE. Gels were transferred to Sequi-Blot™ PVDF Membrane (Bio-Rad, München, Germany) by semi-dry western blotting procedure. We used the following antibodies: P21 Waf1/CIP1 (DCS60) mouse mAB (#2946 Cell Signaling, Danvers, USA), P21 Waf1/CIP1 (DCS60) rabbit mAB (#2947 Cell Signaling), hnRNPK (A222) rabbit AB (# 4699 Cell Signaling), ß-Actin mouse mAB (A5441, Sigma-Aldrich, St. Louis, USA), GAPDH mouse AB (# 9484 Abcam, Milton, UK), P53 mouse mAB clone DO-1 (#P6874 Sigma-Aldrich), phosphoCHK2 (Thr68), rabbit mAB (#2661 Cell Signaling), phosphoCHK1 (Ser345) (133D3) rabbit mAb (#2348, Cell Signaling), anti-mouse IgG, HRP-linked Antibody (#7076 Cell Signaling), anti-rabbit IgG, HRP-linked Antibody (#7074 Cell Signaling). Blots were developed by Western Lightning Plus ECL (Perkin Elmer, Waltham), documented with the ChemiDoc Imager (Bio-Rad) and evaluated with the Image Lab software (version 5.0 build 18, Bio-Rad). The measured intensities were normalized by the housekeeping gene ß-actin, each experiment was repeated at least three times and the relative expression was compared between the four experimental groups on one blot. The relative expression is expressed as percent of the corresponding control (no irradiation, no hnRNPK knockdown).

Colony-forming assay

Forty-eight hours after siRNA transfection, tumor cells were detached by the use of 1 ml accutase. Cell numbers were determined via Casy1® and cell suspensions of 900 and 1800 cells/ml for dosages of 0, 1, 2, 4 and 6, 8 Gy, respectively, were prepared and irradiated in a falcon tube using a Cs137 source. Cell numbers were chosen according to pilot tests to reach between 50 and 500 colonies after treatments. Non-irradiated cultures were processed in parallel and the assay was performed as previously described (23). Colony formation was assessed with the COLCOUNT™ system (Oxford Optronix Ltd., Abingdon, UK). Each experiment was performed in duplicates and repeated at least three times. Survival curves were created.

Immunohistochemistry

Immunohistochemistry was performed as previously described using hnRNP K (R332) rabbit mAB (#4675 Cell Signaling) (23).

Results

To determine the p53 status of the cell lines, we sequenced the p53 gene. While A549 cells bear wildtype p53, we found a mutation in the p53 gene of HNSCCUM-02T cells: the codon 205, exon 6, is changed (TAT -> GAT) which results in an exchange of the amino acid tyrosine for aspartic acid in the DNA-binding domain of the protein (Figure 1A). Taken into account, that we could not detect loss of p53 expression due to the mutation, but on the contrary accumulation of the protein in the cells up to a level, which exceeds the expression in wt-p53 cells (Figure 5), we suggest the classification of the mutation as a gain-of-function (GOF) mutation (24).

Both cell lines bear approximately the same hnRNPK level under resting conditions confirmed by western blot (Figure 1C) and immunohistological staining (Figure 1B). The knockdown of hnRNPK by the use of siRNA reduced the protein level significantly to approximately 21% in A549 and 23% in HNSCCUM-02T cells relative to cells treated with the transfection reagent alone (Figure 1D). A positive control (knockdown of GAPDH) and a negative control with scrambled siRNA had the expected effects (Figure 1E). To exclude side effects of the transfection reagent, control cells were treated with the transfection reagent only (lipofectamine).

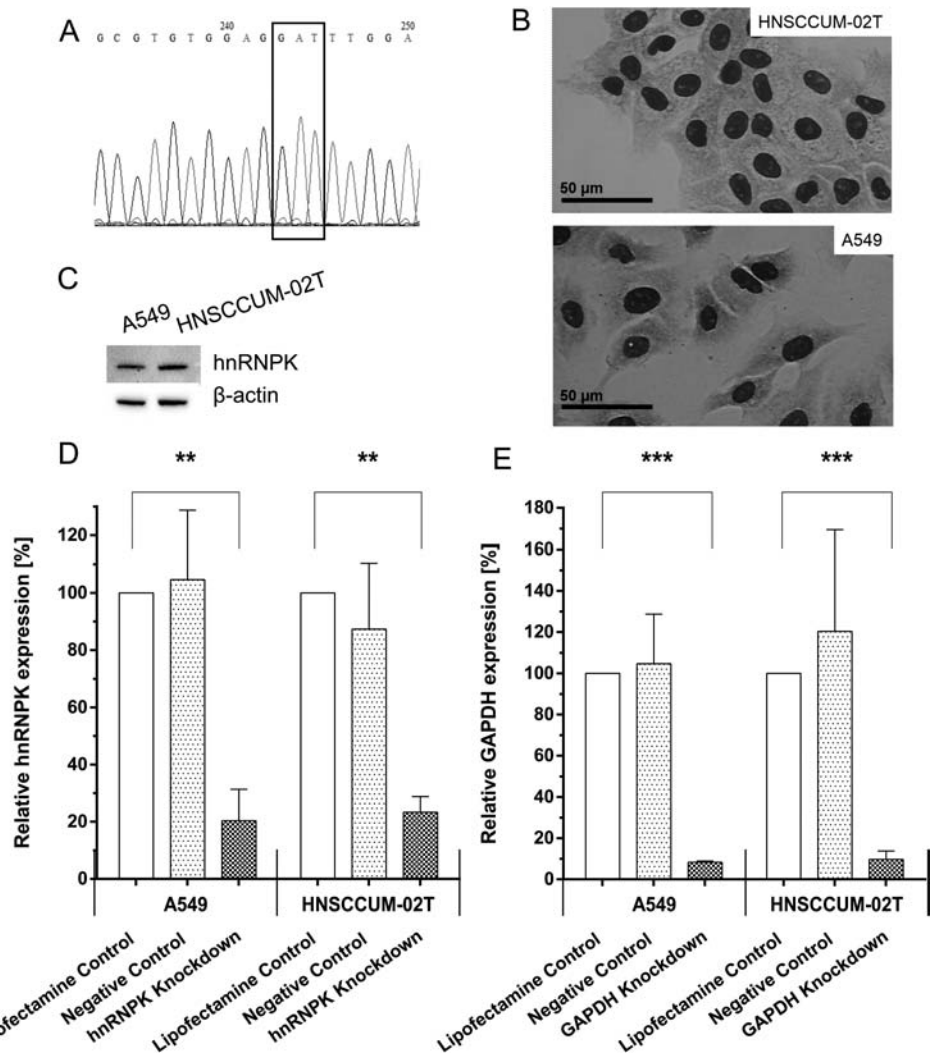


Figure 1. Expression and knockdown of hnRNPK. (**A**) Sequencing revealed a mutation in codon 205 of the p53 gene in HNSCCUM-02T cells resulting in a T to G exchange. (**B**) Immunohistological staining showed comparable nuclear expression levels of hnRNPK in both cell lines under investigation confirmed by western blot analysis (**C**). (**D**) By siRNA transfection a significant reduction of the hnRNPK expression levels was achieved and (**E**) the control (knockdown of GAPDH) also had the expected effect. Shown are means ± SD, $^{**}P < 0.01$, $^{***}P < 0.001$, Student's *t*-test, pairwise comparison, $N = 3$.

HnRNPK knockdown increases γH2AX counts in irradiated cells

We performed the yH2AX assay after knockdown of hnRNPK. The tumor cells were irradiated with the indicated dosages and yH2AX foci were quantified during the following 48 h.

After knockdown of hnRNPK DNA damage levels increased in irradiated A549 and HNSCCUM-02T cells (Figures 2 and 3). The yH2AX foci analyses in A549 cells (wt p53) showed a time and dosage dependent increase of foci numbers after irradiation (Figure 3A, light grey). The maximum foci numbers were reached after 0.5 h in the 2 Gy group and after 1 h in the 4 and 8 Gy groups. Non-irradiated cells showed < 1 foci per cell in all samples. After 48 h, the foci numbers decreased to baseline levels. HnRNPK knockdown further increased DNA damage of irradiated cells (Figure 3A, dotted bars).

Next we analyzed DNA damage in the p53-mutant cell line HNSCCUM-02T. The temporal pattern of γH2AX foci number in irradiated cells was comparable to that of A549 cells (Figure 3B, light grey). The maximum foci numbers per cell were reached after 0.5 h in the 2 and 8 Gy groups and after 1 h in the 4 Gy group. Within 48 h, the foci number returned to baseline.

After knockdown of hnRNPK in HNSCCUM-02T cells, DNA damage levels increased (Figure 3B, dotted bars) compared to cells which received solely irradiation. This increase was higher in numbers compared to the wt-p53 cell line A549. Remarkably, there was a shift in the time course of foci appearance and the subsequent disappearance. The foci numbers raised prolonged and disappearance of the foci after irradiation was delayed. In the 8 Gy group, the number of foci per cell increased until 2 h after irradiation when hnRNPK was knocked down, while the foci number in the controls began to drop after 0.5 h (for a table of gamma-H2AX foci numbers and standard deviations please refer to the Supplementary Materials, available at *Carcinogenesis* online).

HnRNPK is a relevant factor for tumor cell survival

In the light of our finding that knockdown of hnRNPK increased DNA damage and delayed repair after irradiation, we investigated the importance of hnRNPK for the survival of tumor cells after irradiation.

Three different test groups of HNSCCUM-02T and A549 cells (untreated controls, lipofectamine controls and knockdown of hnRNPK) were irradiated with the indicated dosages and their

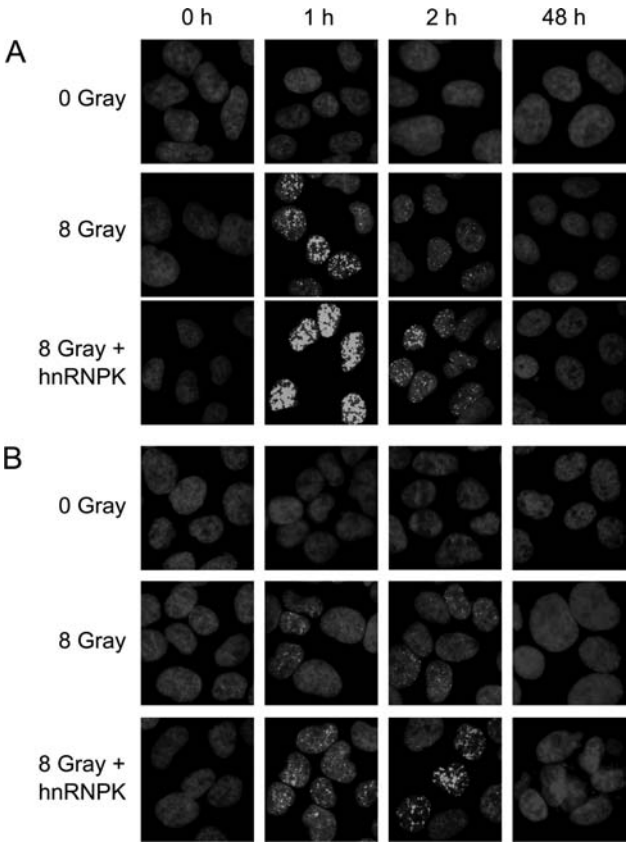


Figure 2. γH2AX assay of HNSCCUM-02T and A549 cells. Shown are exemplary images of γH2AX foci (green) in the nuclei (blue) after irradiation and/or hnRNPK knockout in A549 (**A**) and HNSCCUM-02T (**B**) cells. HnRNPK knockdown increased foci numbers initiated by irradiation.

colony-forming capability was measured. The results show a reduced colony formation of cells transfected with siRNA against hnRNPK after irradiation compared to the control cells in both cell lines. The surviving fraction of A549 cells transfected with siRNA was increasingly depending on the irradiation dosage. HnRNPK knockdown alone did not affect the survival of A549 cells significantly (Figure 4A). HNSCCUM-02T cells also showed reduced colony formation after treatment with siRNA against hnRNPK alone without additional irradiation. The reduction in survival after irradiation became more prominent in a dose dependent manner (Figure 4B).

Analysis of DNA damage signaling

To gain insights into the role of hnRNPK in DDR signaling, we analyzed the expression levels of p21, p53 and the phosphorylation of CHK1 and CHK 2 15 min, 1, and 4 h after 8 Gy irradiation of the cells, respectively. Irradiated and siRNA treated cells served as controls. A549 cells showed rising p53 expression levels after irradiation in a time-dependent manner as well as cells with hnRNPK knockdown (Figure 5A). HNSCCUM-02T showed higher p53 expression in non-irradiated cells compared to A549 cells and we could not detect any increase of p53 levels after irradiation. After knockdown of hnRNPK, the p53 level of HNSCCUM-02T cells was slightly reduced irrespective of irradiation (Figure 5B).

In line with p53 expression, also the p21 expression increased in A549 cells four hours after irradiation. The knockdown of hnRNPK was not able to inhibit the p21 response, but it was attenuated slightly although not statistically significant (Figure 5C). In HNSCCUM-02T cells, no increase of p21 expression was detectable after irradiation. There was a more or less stable level of p21 expression unaffected by knockdown of hnRNPK and irradiation (Figure 5D). Compared to the p21 level in A549 cells after irradiation, HNSCCUM-02T cells exhibited a very low p21 expression 4 h after irradiation (Figure 5C and D).

We were able to show that the phosphorylation of CHK1 and CHK2 is an early and rapid response upon irradiation in both A549 and HNSCCUM-02T cells (Figure 6). Within 15 min after irradiation, the pCHK1 and pCHK2 level in the cells rose compared to non-irradiated control cells. Within 4 h after irradiation, the level of phosphorylated CHK1 and CHK2 returned to baseline (Figure 6B). Knockdown of hnRNPK had no influence on the phosphorylation of CHK1 and CHK2.

Discussion

We analyzed the impact of hnRNPK knockdown on the DDR after gamma irradiation and found that hnRNPK knockdown increases DNA damage levels in irradiated cells, especially in those with mutated p53. They showed increased damage levels compared to wt-p53 cells and delayed repair as well as reduced survival.

HnRNPK is involved in many cellular processes, is overexpressed in different tumor entities and serves as a cofactor of p53 (8,25), which plays an important role in the DDR. The capacity of tumor cells to repair DNA damage is critical for the patients' outcome after radiotherapy, and p53 and its cofactors have a decisive influence hereupon (26–28).

The γH2AX foci analysis showed increased DNA damage upon irradiation after knockdown of hnRNPK in both cell lines. Additionally, the time course of appearance and disappearance of foci was altered in the p53-mutant cell line. After knockdown of hnRNPK, the number of foci per cell continued to rise for a longer period compared to cells, which were subjected to irradiation alone. We suggest that the apparent increase in DSB levels is most likely attributable to slowed repair kinetics. The hnRNP family is involved in gene expression at various levels (29) also in the context of DDR (30,31). This underlines the importance of hnRNPK in the light of radiation resistances, since repair processes of tumor cells decisively influences the outcome after radiotherapy (32). However, the overall repair capacity of tumor cells is not affected by knockdown of hnRNPK. After 48-h irradiation, the number of foci returned to baseline, which is in line with results of Eder *et al.* (33).

Furthermore, we could observe reduced colony-forming capability of tumor cells after knockdown of hnRNPK and irradiation. This observation is supported by findings made in a pancreatic and a malignant melanoma cancer cell lines (33,34). It supports the idea, that loss of hnRNPK leads to cell death (apoptosis) (35,36) especially under conditions of distress (37) such as irradiation. The reduction of the surviving fraction was much more pronounced in the cell line with defective p53.

Both analyses show that hnRNPK is essential for the DDR and the survival of tumor cells after irradiation. Especially cells with mutant p53 seem to be more sensitive to hnRNPK knockdown, as the colony-forming ability of non-irradiated cells after hnRNPK knockdown was only reduced in the cell line with mutant p53. Potentially tumor cells with gain of function mutation of p53 are particularly dependent on the stabilization of p53 via hnRNPK (16) because they entail reduced stability of the protein (38) which has to be compensated. However, this hypothesis remains to be proved.

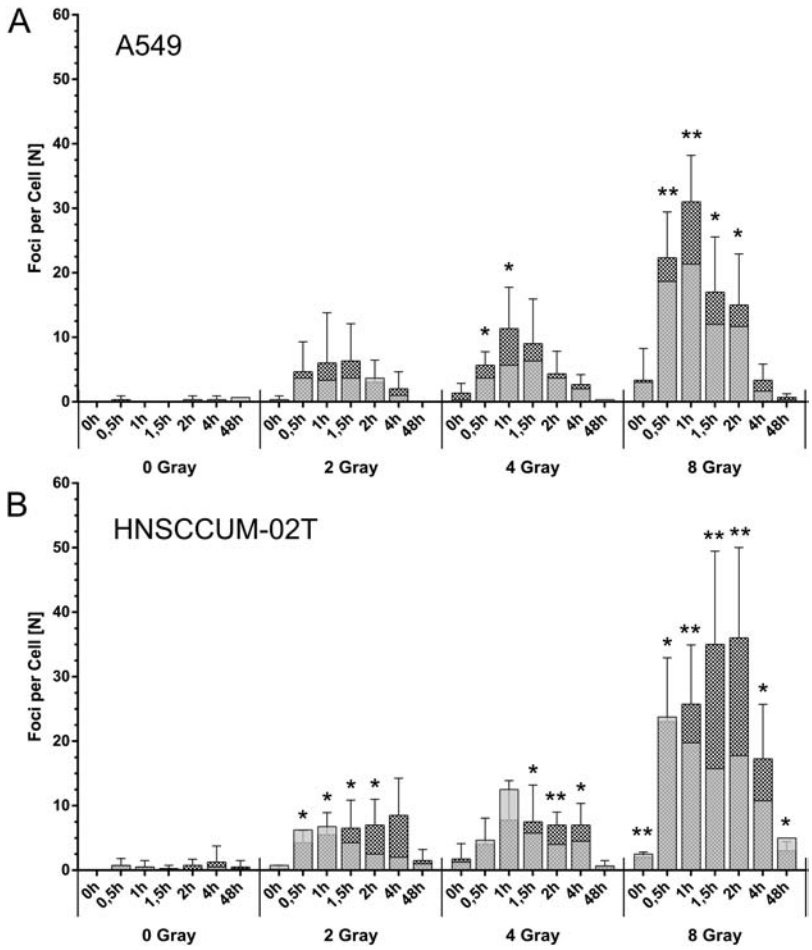


Figure 3. yH2AX foci after irradiation and hnRNPK knockdown. Plotted in light grey are the yH2AX foci numbers in control cells treated with lipofectamine alone. The dotted bars show the numbers of yH2AX foci in hnRNPK-siRNA transfected cells. After knockdown, the number of yH2AX foci after irradiation was increased in A549 (**A**) and HNSCCUM-02T (**B**) compared to cells that experienced irradiation alone. Shown are means ± SD, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, Student's *t*-test, pairwise comparison between irradiated cells and non-irradiated controls, $N = 3$. Statistical analysis by fitting a linear mixed model to the log (foci per cell) with treatment, radiation dose and time as covariates was not able to find statistically significant changes due to high SD.

Domselaar *et al*. could show reduced cellular viability of HeLa cells after hnRNPK knockdown. Furthermore, they showed that hnRNPK knockdown rendered tumor cells more sensitive to cytotoxic lymphocyte-mediated killing and hnRNPK being a substrate of all granzymes. These findings emphasize that downregulation of hnRNPK could lead to increased cell death and support the idea that overexpression in tumor cells could represent an escape mechanism, which leads to poor prognosis due to treatment failure (6,10,11,37,39). This also renders hnRNPK interesting as prognostic marker (39).

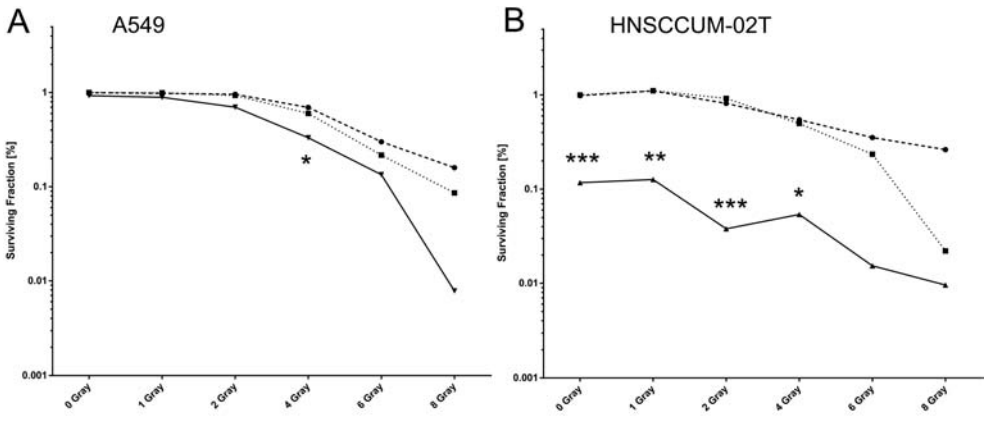
Up to now, the nature of this escape mechanism via hnRNPK upregulation remains elusive, but considering the importance of p53 in tumorigenesis and the fact that hnRNPK serves as its cofactor, we suppose that the interaction between both proteins is of relevance in this context.

Knockdown of hnRNPK resulted in reduced p21 levels in A549 cells compared to control cells, i.e., the p53-p21 axis is perturbed in case of reduced hnRNPK levels. Reduced p21 levels after irradiation could be one reason for the reduced survival of these cells after irradiation and hnRNPK knockdown, since the inhibition of cell cycle progression by p21 allows tumor cells to repair sustained DNA damage while apoptosis is inhibited (40).

In the p53-mutated cell line—HNSCCUM-02T—there was no p21 induction observed after irradiation and the hnRNPK knockdown had no effect on the p21 expression. This fits to the observation of no upregulation of p53 after irradiation. It can be hypothesized, that the mutation in the DNA-binding domain of p53 hampers binding of the protein to DNA and thus prevents its normal function. It is also remarkably that HNSCCUM-02T cells show a very high p53 expression in non-irradiated cells, which also points to a malfunction of p53 in this cell line, possibly a gain of function mutation (24). To what extent hnRNPK is able to interact with mutant p53, is not fully understood. Zhou *et al*. (34) could show different interactions between hnRNPK and mutant p53 depending on the mutation.

The knockdown of hnRNPK had no effect on the phosphorylation of CHK1 and CHK2, which is in line with the idea that phosphorylation of the checkpoint kinases precedes hnRNPK and p53 activation (Figure 7).

In summary, we found more yH2AX foci in both lines after combined knockdown of hnRNPK and gamma irradiation in a dose-dependent manner. Moreover, the colony-forming capability of the tumor cells was reduced after hnRNPK knockdown. We hypothesize that knockdown of hnRNPK might represent a possibility to radio sensitize tumor cells, especially those carrying mutant p53. The elevated DNA damage levels could lead to enhanced apoptosis of tumor cells and finally a better outcome of patients, who received drugs



C

	A549									HNSCCUM-02T								
	Untreated Control			Lipofectamine Control			hnRNPK Knockdown			Untreated Control			Lipofectamine Control			hnRNPK Knockdown		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
0 Gray	0.998	0.021	5	1.000	0.000	5	0.927	0.156	5	0.984	0.143	4	1.000	0.000	4	0.117	0.087	4
1 Gray	0.983	0.027	5	0.996	0.042	5	0.889	0.231	5	1.107	0.105	4	1.110	0.129	4	0.126	0.133	4
2 Gray	0.957	0.087	5	0.932	0.067	5	0.700	0.266	5	0.815	0.389	4	0.920	0.034	4	0.038	0.025	4
4 Gray	0.695	0.256	5	0.600	0.155	5	0.331	0.185	5	0.547	0.351	4	0.498	0.127	4	0.054	0.077	4
6 Gray	0.300	0.154	5	0.216	0.142	5	0.134	0.172	5	0.353	0.265	4	0.235	0.188	4	0.015	0.022	4
8 Gray	0.159	0.168	5	0.086	0.070	5	0.008	0.006	5	0.262	0.207	4	0.022	0.011	4	0.010	0.005	4

Figure 4. Colony forming assay. Shown are survival curves of A549 (**A**) and HNSCCUM-02T (**B**) cells after irradiation. In A549 cells, we found significantly reduced surviving fractions after irradiation with 4 Gy combined with knockdown of hnRNPK compared to control cells. In HNSCCUM-02T cells, the surviving fraction in non-irradiated cells and cells irradiated with 1–4 Gy, respectively, was significantly lower compared to control cells. Dotted line: lipofectamine controls, dashed line: untreated controls and solid line: hnRNPK knockdown cells. Shown are means. In (**C**), means ± SD are listed.

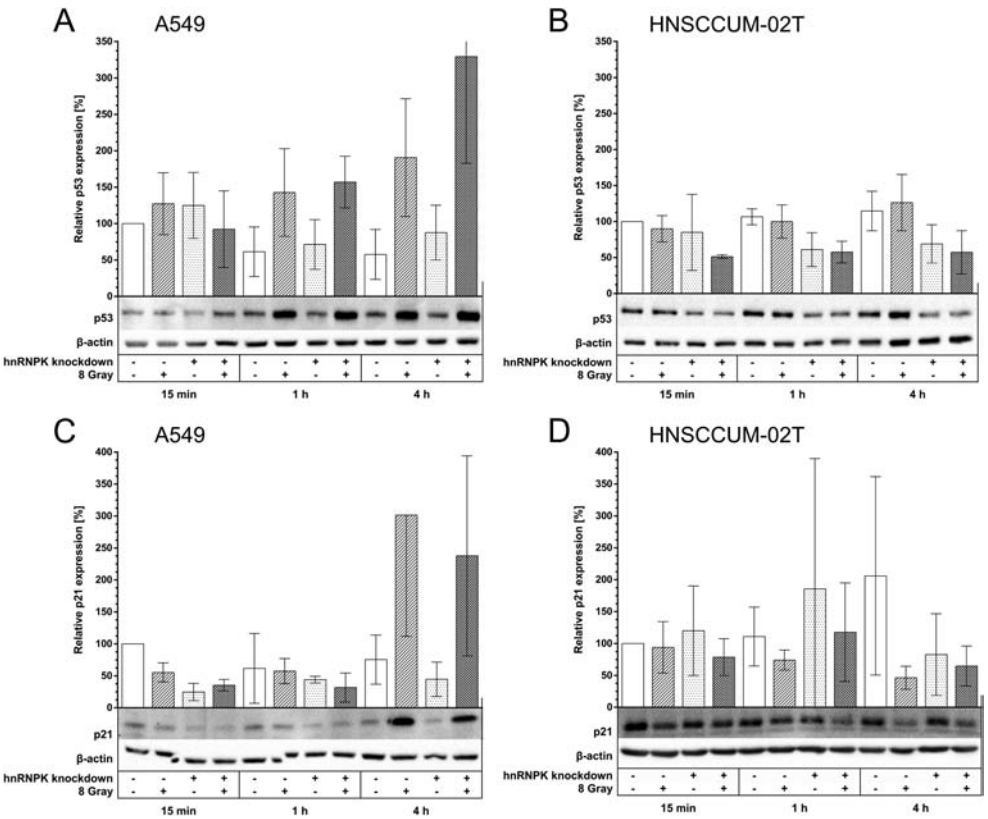


Figure 5. p53 and p21 expression in A549 and HNSCCUM-02T cells after irradiation and knockdown of hnRNPK. (**A**) In A549 cells, the p53 expression was rising from 15 min to 4 h after irradiation as well as in cells with additional hnRNPK knockdown. (**B**) HNSCCUM-02T cells showed higher expression levels of p53 in non-irradiated cells compared to A549. While A549 cells showed an increase in p53 expression upon irradiation, p53 levels remained unchanged in HNSCCUM-02T cells. After knockdown of hnRNPK, the p53 levels of HNSCCUM-02T cells were slightly reduced with and without irradiation. (**C**) and (**D**) Four hours after irradiation the p21 level was clearly elevated in A549, while remaining unchanged in HNSCCUM-02T. The knockdown of hnRNPK resulted in no significant changes of p21 expression in both cell lines. Shown are means ± SD, *N* = 3.

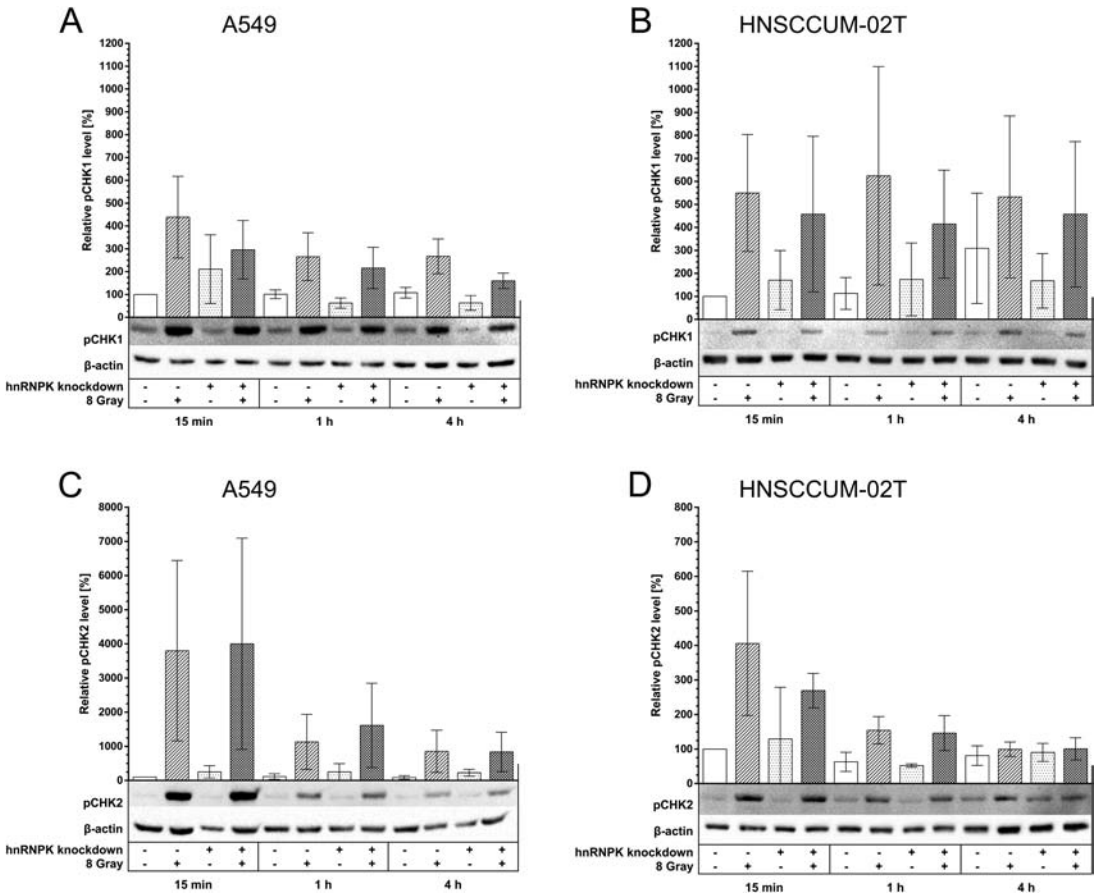


Figure 6. Phosphorylation of CHK1 and CHK2 in A549 and HNSCCUM-02T cells after irradiation. (**A**) In A549 cells as well as in (**B**) HNSCCUM-02T cells phosphorylation of CHK1 increased immediately after irradiation. The phosphorylation of CHK2 also increased in both cell lines (**C** and **D**) and slowly returned to baseline within 4 h after irradiation. Knockdown of hnRNPK had no influence on the phosphorylation of CHK1 and CHK2. Shown are means ± SD, N = 3.

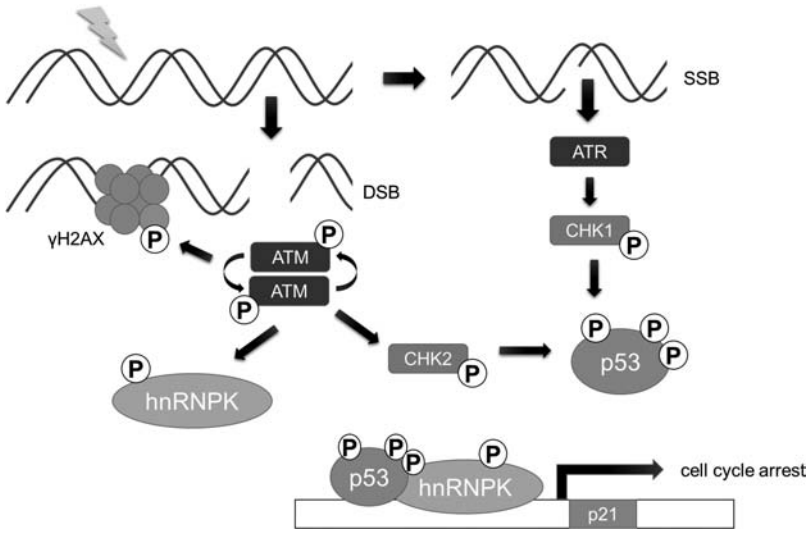


Figure 7. Cartoon of the DNA damage response upon single strand (SSB) and double strand breaks (DSB). In response to DNA damage, the kinases ataxia telangiectasia mutated and ataxia telangiectasia (ATM) and Rad3-related protein (ATR) are activated. They phosphorylate histone H2AX as well as the signaling transducers CHK1 and CHK2. This finally leads to the phosphorylation and stabilization of hnRNPK and p53. Consequently, they can transcriptionally activate e.g. p21 and other DNA damage repair associated genes, resulting in cell cycle arrest and DNA repair.

inhibiting hnRNPK and radiotherapy. Therefore, we recommend hnRNPK for further studies evaluating its potential as a drug target to be used in a future adjuvant therapeutic setting.

Supplementary material

Supplementary data are available at *Carcinogenesis* online.

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