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Original Articles

Sensitizing tumor cells to radiation by targeting the heat shock response

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ABSTRACT

Elevated levels of heat shock proteins (HSPs) contribute to tumor cell survival and mediate protection against radiation-induced cell death. Hsp90 inhibitors are promising radiosensitizers but also activate heat shock factor 1 (HSF1) and thereby induce the synthesis of cytoprotective Hsp70. In this study the heat shock response inhibitor NZ28 either alone or in combination with the Hsp90 inhibitor NVP-AUY922 was investigated for radiosensitizing effects, alterations in cell cycle distribution and effects on migratory/invasive capacity of radioresistant tumor cells. NZ28 reduced the constitutive and NVP-AUY922-induced Hsp70 expression by inhibition of the HSF1 activity and inhibited migration and invasion in human lung and breast tumor cells. Treatment of tumor cells with NZ28 significantly increased their radiation response. One possible mechanism might be a decrease of the radioresistant S-phase. When combined with the Hsp90 inhibitor NVP-AUY922 the concentration of NZ28 could be significantly reduced (1/10th–1/20th) to achieve the same radiosensitization. Our results demonstrate that a dual targeting of Hsp70 and Hsp90 with NZ28 and NVP-AUY922 potentiates the radiation response of tumor cells that are otherwise resistant to ionizing radiation.

Introduction

The major stress-inducible molecular chaperone, heat shock protein 70 (Hsp70, Hsp70A1A), fulfills a variety of housekeeping and cytoprotective functions. Normal cells constitutively express low amounts of Hsp70. Following a broad variety of different stress stimuli (e.g. heat shock, Hsp90 inhibition) the expression of Hsp70 is strongly increased. The main factor which is involved in the transcription of Hsp70 is heat shock factor 1 (HSF1). After trimerization HSF1 translocates to the nucleus and binds to the heat shock element (HSE) in the promoter region of Hsp70. HSF1 activation is regulated by posttranslational modifications such as phosphorylation, sumoylation and deacetylation [1].

In contrast to normal cells, HSF1 and Hsp70 are highly overexpressed in tumor cells already under physiological conditions and thus contribute to tumor cell survival, migration, invasion and angiogenesis [1–6]. High HSF1 and Hsp70 levels are associated with poor prognosis, metastasis and therapy resistance [1,7,8]. Consequently, a knock-down of HSF1 or Hsp70 results in an increased radiation-induced cell killing [9–12]. The small molecular weight inhibitor NZ28 was found to reduce HSF1 and Hsp70 levels and therefore is meant to exert effects similar to a HSF1 depletion [13,14].

Apart from HSF1/Hsp70, Hsp90 is an attractive anticancer target since Hsp90 chaperones a number of oncogenic client proteins (e.g. HER2, mutant EGFR, AKT, BCR-ABL, survivin, mutant p53, HIF-1α, MMP2, hTERT). Several Hsp90 inhibitors are currently tested in clinical trials. By a simultaneous degradation of multiple oncogenic client proteins, Hsp90 inhibitors reduce tumor cell proliferation and enhance the radiosensitivity of tumor cells [15,16]. However, a negative side effect of Hsp90 inhibition is the activation of HSF1 and subsequently the induction of Hsp70. Therefore, a down-regulation or inhibition of HSF1 or Hsp70 increases the sensitivity of tumor cells toward Hsp90 inhibitors [14,17–20]. Herein, we studied the role of the heat shock response inhibitor NZ28 either alone or in combination with the Hsp90 inhibitor NVP-AUY922 on the activation of HSF1, Hsp70 expression, migration, invasion and radiosensitivity of radioresistant human tumor cell lines.

Abbreviations: Hsp, heat shock protein; HSF1, heat shock factor 1; HSE, heat shock element; SER, sensitizing enhancement ratio.

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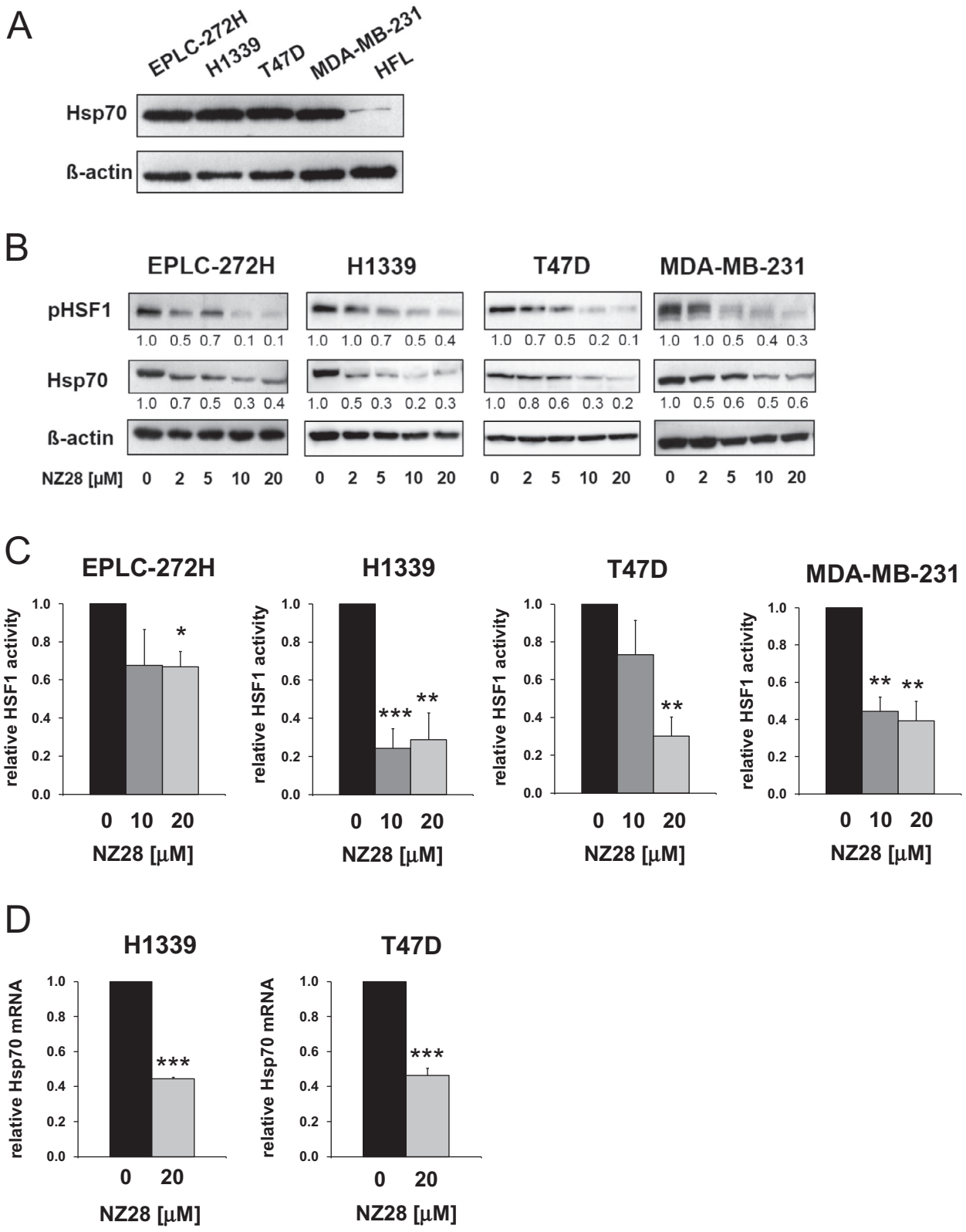

Fig. 1. NZ28 reduces HSF1 activity and Hsp70 expression in tumor cells. (A) Representative immunoblot showing the constitutive expression of Hsp70 in EPLC-272H, H1339, T47D and MDA-MB-231 tumor cells and human fetal lung fibroblasts (HFL). (B) Representative HSF1 phospho S326 (pHSF1) and Hsp70 immunoblots of EPLC-272H, H1339, T47D and MDA-MB-231 tumor cells that were treated for 24 h with different concentrations of NZ28. DMSO (0.2%) treated cells served as control (0 µM NZ28). The protein bands were quantified by densitometry using ImageJ. Numbers under the lanes represent the expression levels of pHSF1 or Hsp70 relative to β-actin. The value of control cells was set to 1 for each cell line. (C) Luciferase assay of EPLC-272H, H1339, T47D and MDA-MB-231 tumor cells transfected with a HSF1 responsive firefly luciferase construct and treated with 10 or 20 µM NZ28 for 24 h. The luciferase activity of vehicle (0.2% DMSO) treated cells (0 µM NZ28) was set to 1. Data are expressed as mean ± SEM of at least 3 independent experiments (**$p \le 0.01$, **$p \le 0.01$, ***$p \le 0.001$). (D) The Hsp70 mRNA expression in H1339 and T47D cells treated with 20 µM NZ28 or 0.2% DMSO (0 µM NZ28) for 24 h was quantified by qRT-PCR. The mRNA levels were normalized to the housekeeping gene β-actin. Data are expressed as mean ± SEM of 3 independent experiments (***$p \le 0.01$).

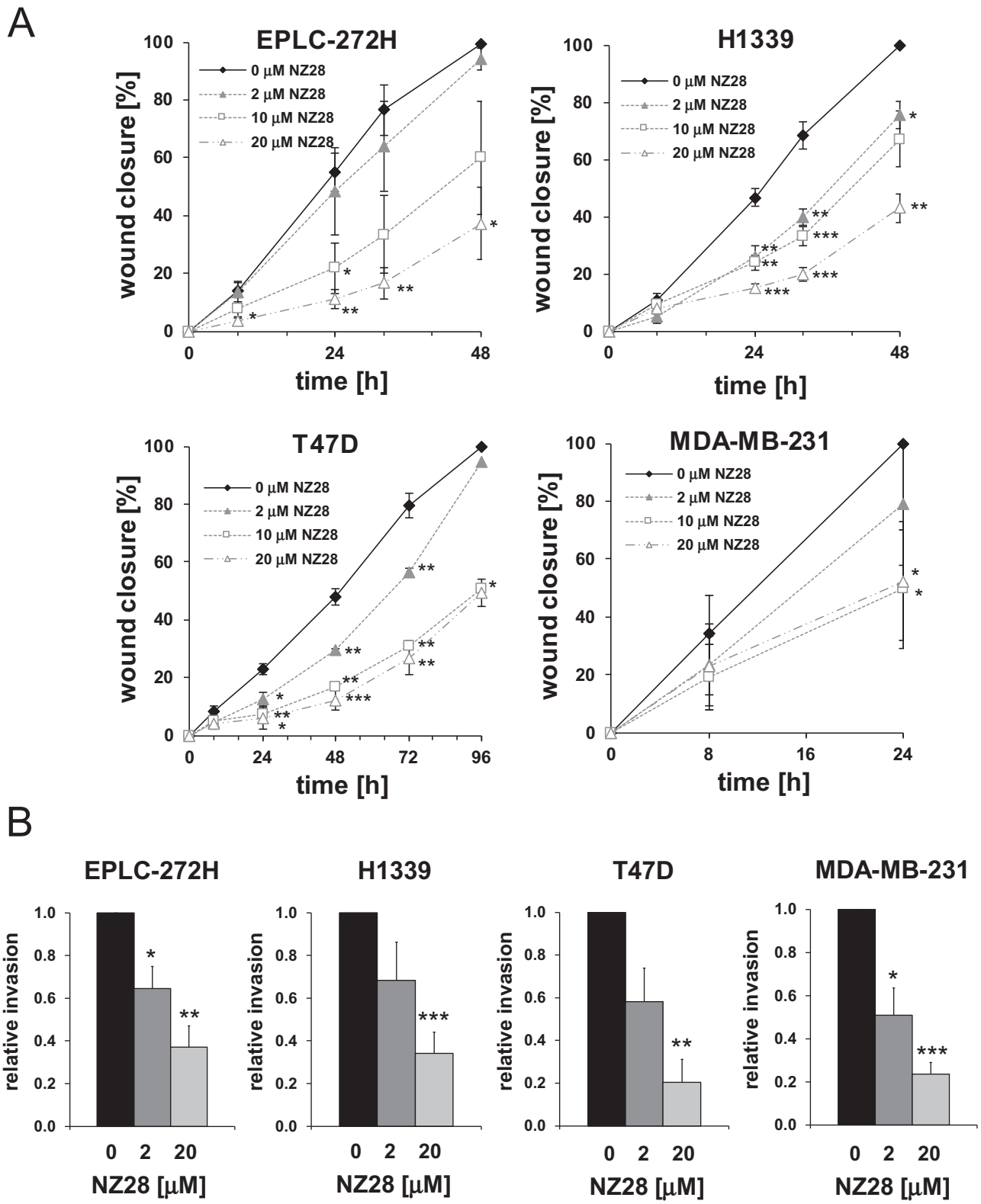

Fig. 2. NZ28 inhibits migration and invasion. (A) Migratory capacity was assessed by a wound healing assay. EPLC-272H, H1339, T47D and MDA-MB-231 tumor cells were plated in culture-inserts and 24 h later, inserts were removed and cells were treated with 2, 10 and 20 μM NZ28 (0 h). 0.2% DMSO diluted in PBS was used as control (0 μM NZ28). The percentage of wound closure was quantified at the indicated time points. Data are expressed as mean ± SEM of 3 independent experiments (*$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$). (B) The invasive capacity of EPLC-272H, H1339, T47D and MDA-MB-231 tumor cells treated with 2 and 20 μM NZ28 for 24 h in relation to vehicle (0.2% DMSO) treated cells was determined by a transwell Matrigel invasion assay. Data are expressed as mean ± SEM of 4 independent experiments (*$p \leq 0.05$, **$p \leq 0.01$***, $p \leq 0.001$).

Materials and methods

Reagents and treatment

10 mM stock solutions of NZ28 (M. Sherman; Boston University School of Medicine, USA) and NVP-AUY922 (Novartis) were prepared in 100% DMSO. Dilutions were performed in PBS. A vehicle control with the respective amount of DMSO diluted in PBS (maximal 0.2%) was used in all experiments. If not indicated otherwise, cells were incubated for 24 h with NZ28 and/or NVP-AUY922.

Cells and cell culture

The human lung (H1339 and EPLC-272H) and breast (MDA-MB-231 and T47D) cancer cell lines and human fetal lung fibroblasts (HFL) (kindly provided by Prof. Rodemann) were cultured as described previously [15,21,22]. The authenticity of the tumor cell lines was tested by the DSMZ (German Collection of Microorganisms and Cell Cultures). Cells were routinely checked and determined as negative for mycoplasma contamination.

Western blot analysis

Cells were lysed as described previously [23]. On immunoblots, proteins were detected with antibodies against Hsp70 (ADI-SPA-810, Enzo Life Sciences), HSF1 phospho S326 (pHSF1) (ab76076, abcam) and β-actin (A5316, Sigma-Aldrich). The protein bands were quantified by densitometry using ImageJ. The expression levels of pHSF1 or Hsp70 were calculated relative to β-actin.

HSE luciferase assay

In order to determine the HSF1 activity, cells were transfected with a HSE reporter plasmid that contains a HSF1 responsive firefly luciferase construct (Qiagen). On day one after transfection, cells were treated with NZ28 for 24 h. The luciferase activity was measured using the Dual Glo Luciferase assay system (Promega). A constitutive Renilla luciferase construct served as an internal control.

Quantitative real-time PCR

To investigate the mRNA expression of Hsp70, RNA was isolated with the RNeasy Mini Kit (Qiagen) and reverse transcription of RNA was performed with the QuantiTect Reverse Transcription Kit (Qiagen) according to the manufacturer's instructions. qRT-PCR was performed in a LightCycler 480 (Roche) by using the QuantiTect SYBR Green PCR Kit (Qiagen). Primers used for qRT-PCR were as follows: ACTB-F: GACGACATGGAGAAAATCTG, ACTB-R: ATGATCTGGGTCATCTTCTC, HspA1A-F: AATTTCCTGTGTTTGCAATG, HspA1A-R: AAAATGGCCTGAGTTAAGTG. Each sample was measured in triplicate and the mean Ct was calculated. Relative expression was calculated using the ΔΔCt method. The mRNA expression of β-actin was used as an internal control.

Wound healing assay

To determine the migratory capacity of tumor cells, wound healing assays were performed. Cells were plated in culture-inserts (Ibidi) and 24 h later, inserts were removed and cells treated with 2, 10 and 20 μM NZ28 (0 h). 0.2% DMSO diluted in PBS was used as control (0 μM NZ28). After 24 h the medium was replaced by fresh medium without NZ28 or DMSO. The migration of cells into the cell-free gap was monitored microscopically and quantified using ImageJ's wound healing tool.

Matrigel invasion assay

Invasion of tumor cells was measured by transwell Matrigel assay. Cells were seeded in 6 cm dishes and 24 h later treated with 2 and 20 μM NZ28. 0.2% DMSO diluted in PBS was used as control (0 μM NZ28). After 24 h, cells were trypsinized, counted and plated in Biocoat™Matrigel® Invasion chambers (Corning) in serum-free medium. 10% FCS-containing medium was added as a chemo-attractant to the wells in the bottom. Transmigrated cells were fixed after 24 h, stained with DAPI and counted. The number of NZ28-treated cells that migrated through the matrigel was calculated in relation to vehicle treated (0.2% DMSO) cells (0 μM NZ28).

Clonogenic assay and irradiation

To measure the radiosensitivity, clonogenic assays were performed as described previously [15]. The cells were seeded in 12-well plates, one day later treated with NZ28/NVP-AUY922 and 24 h later irradiated using the RS225A irradiation device (Gulmay Medical Ltd) at a dose rate of 1 Gy/min (70 keV). After irradiation the medium was exchanged by a drug-free medium. On day 9 (H1339) or 16 (T47D) after seeding, colonies were fixed, stained and counted. Survival curves were fitted to the linear quadratic model using Sigmaplot (Systat Software Inc).

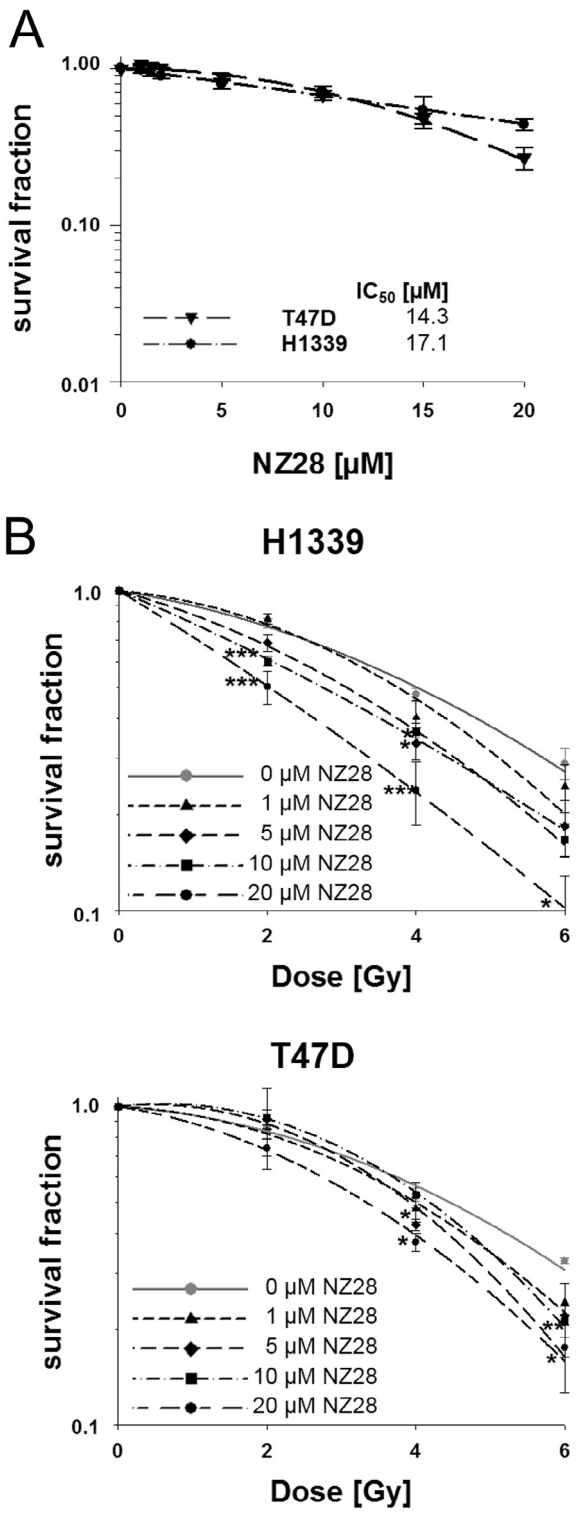

Fig. 3. NZ28 increases radiosensitivity of tumor cells. Clonogenic survival of cells treated with NZ28 (A) or with NZ28 and irradiation (B). (A) One day after seeding H1339 and T47D cells were treated with different concentrations of NZ28. DMSO (0.2%) treated cells served as control. After 24 h the medium was replaced by a drug-free medium. Colonies were fixed either 9 (H1339) or 16 (T47D) days after seeding. The survival fractions were calculated relative to the DMSO-treated control. IC_{50} (half maximal inhibitory concentration) values are indicated. (B) One day after seeding H1339 and T47D cells were treated with NZ28 for 24 h, then irradiated and immediately after irradiation fresh medium without NZ28 was added. DMSO treated cells served as control (0 μM NZ28). Colonies were fixed either 9 (H1339) or 16 (T47D) days after seeding. The survival fractions were calculated after normalization for cell killing by NZ28 alone. Data are expressed as mean ± SEM of at least 3 independent experiments ($^{*}p \le 0.05$, $^{**}p \le 0.01$, $^{***}p \le 0.001$).

Cell cycle analysis

To analyze the cell cycle distribution, cells were fixed, stained with propidium iodide in the presence of RNase and analyzed on a FACSCalibur flow cytometer (BD Biosciences) as described previously [24]. The cell cycle distribution was calculated using Modfit software (Verity Software House Inc).

Statistics

Statistical analysis was performed using SPSS 18.0.2 software (IBM). The Student's t-test was used to evaluate significant differences (*$p \le 0.05$, **$p \le 0.01$, ***$p \le 0.001$). All experiments were independently performed at least 3 times.

Results

NZ28 decreases Hsp70 levels and inhibits activation of HSF1

As shown in Fig. 1A, lung (EPLC-272H, H1339) and breast (T47D, MDA-MB-231) cancer cell lines exhibited high constitutive Hsp70 levels in comparison to normal human fetal lung fibroblasts (HFL). Knock-down of HSF1 revealed that the high constitutive Hsp70 expression levels in H1339 tumor cells depend on HSF1 [25].

Following treatment with different concentrations of the small molecule inhibitor NZ28 [13,14] the Hsp70 levels decreased in all 4 cancer cell lines (Fig. 1B). In parallel, the amount of HSF1 phosphorylated at serine 326 (pHSF1 Ser326) was down-regulated, which indicates that the HSF1 activation was reduced by NZ28 (Fig. 1B) [26]. The inhibitory effect of 10 and 20 μM NZ28 on the HSF1 activity was confirmed in EPLC-272H, H1339, T47D and MDA-MB-231 tumor cells transfected with a HSE reporter luciferase gene (Fig. 1C). Quantitative RT-PCR was performed to address the question of whether inhibition of HSF1 by NZ28 affects the transcription of Hsp70. In line with the reduced HSF1 activity, NZ28 significantly down-regulated Hsp70 mRNA expression in H1339 and T47D tumor cells (Fig. 1D). These data suggest that NZ28 inhibits the basal HSF1 activity and down-regulates constitutive Hsp70 transcription in tumor cells.

NZ28 inhibits tumor cell migration and invasion

Hsp70 and HSF1 were found to be associated with the migratory capacity of tumor cells [2,4,6,11]. Therefore, we examined whether NZ28 affects tumor cell migration. In wound healing assays we could show that NZ28 significantly inhibited the migration of lung and breast cancer cell lines in a concentration-dependent manner (Fig. 2A, Supplementary Fig. S1). In addition to migration, the invasive capacity through Matrigel was also significantly inhibited by NZ28 in all 4 cell lines (Fig. 2B).

NZ28 increases the radiosensitivity

A comparison of the intrinsic radiosensitivity of the 4 different tumor cell lines revealed that H1339 and T47D cells are the two most radioresistant tumor cell lines (indicated by the highest D_{50} values) (D_{50}: EPLC-272H, 3.7 Gy; H1339, 4.0 Gy; T47D, 4.5 Gy; MDA-MB-231, 3.2 Gy). Therefore, H1339 and T47D cells were chosen to test the radiosensitizing effect of NZ28. NZ28 alone similarly affected the clonogenic survival of T47D and H1339 cells with IC_{50} (half maximal inhibitory concentration) values of 14.3 and 17.1 μM, respectively (Fig. 3A). When combined with irradiation, concentrations above 5 μM NZ28 significantly reduced the survival fraction of both cell lines (Fig. 3B, Table 1). The radiosensitizing effect of NZ28 was confirmed by calculating the sensitizing enhancement ratios (SER) which were 1.99 in H1339 and 1.34 in T47D cells treated with 20 μM NZ28 (Table 1).

For a better understanding of the mechanisms that result in the NZ28-mediated radiosensitization, apoptosis, DNA double strand breaks and cell cycle distribution were analyzed. Neither radiation-induced apoptosis (Caspase-3, 24 h post irradiation) nor the amount of residual DNA double strand breaks (γH2AX/p53BP1 foci, 24 h post irradiation) were significantly affected by a treatment with 20 μM NZ28 (data not shown). In contrast, NZ28 significantly reduced the fraction of tumor cells in the radioresistant S-phase from 33.8% to 25.5% in H1339 and from 10.5% to 4.0% in T47D cells (Fig. 4).

A combined treatment with low doses of NZ28 and NVP-AUY922 potentiates the radiosensitivity of tumor cells

We have previously shown that a continuous treatment of H1339 cells with the Hsp90 inhibitor NVP-AUY922 can enhance the radiosensitivity but also increases the expression of Hsp70 [15]. Herein, we investigated whether NZ28 can inhibit the NVP-AUY922-induced activation of HSF1 and the expression of Hsp70 to increase the NVP-AUY922-mediated radiosensitization. As shown in Fig. 5, NZ28 reduced the NVP-AUY922-induced activation of HSF1 (as determined by a phosphorylation of serine 326) and the expression of Hsp70. As a consequence, a combined treatment of tumor cells with low concentrations of NZ28 (1 μM) and NVP-AUY922 (2 nM)

Table 1
Summary of radiobiological parameters depicted in Figs. 3B and 6.

	SF_2[a]	SF_4[a]	SF_6[a]	α [Gy^{-1}][b]	β [Gy^{-2}][b]	D_{50} [Gy][c]	SER[d]
H1339							
Control	0.79 ± 0.03	0.47 ± 0.02	0.29 ± 0.03	0.085	0.022	4.01	1.00
1 μM NZ28	0.82 ± 0.03	0.40 ± 0.05	0.24 ± 0.04	0.049	0.037	3.74	1.07
5 μM NZ28	0.69 ± 0.04	0.33 ± 0.04**	0.18 ± 0.04	0.103	0.026	3.18	1.18
10 μM NZ28	0.60 ± 0.02***	0.36 ± 0.02*	0.17 ± 0.02	0.179	0.013	2.73	**1.47**
20 μM NZ28	0.50 ± 0.06***	0.24 ± 0.05***	0.10 ± 0.03*	0.225	0.010	2.01	**1.99**
2 nM NVP-AUY922	0.75 ± 0.06	0.42 ± 0.02	0.22 ± 0.03	0.101	0.026	3.56	1.12
1 μM NZ28 + 2 nM NVP-AUY922	0.64 ± 0.04*	0.32 ± 0.02**	0.17 ± 0.04	0.191	0.019	2.83	**1.41**
T47D							
Control	0.86 ± 0.06	0.53 ± 0.03	0.33 ± 0.01	0.035	0.027	4.46	1.00
1 μM NZ28	0.83 ± 0.04	0.48 ± 0.04	0.24 ± 0.04	0.024	0.037	4.00	1.12
5 μM NZ28	0.91 ± 0.06	0.43 ± 0.02*	0.22 ± 0.06	–0.057	0.060	3.92	1.14
10 μM NZ28	0.92 ± 0.22	0.53 ± 0.05	0.21 ± 0.02**	–0.067	0.055	4.19	1.06
20 μM NZ28	0.74 ± 0.11	0.38 ± 0.02*	0.17 ± 0.05*	0.083	0.037	3.34	**1.34**
2 nM NVP-AUY922	0.81 ± 0.06	0.44 ± 0.02*	0.15 ± 0.02*	–0.001	0.052	3.64	**1.22**
1 μM NZ28 + 2 nM NVP-AUY922	0.78 ± 0.03	0.28 ± 0.01***	0.11 ± 0.01***	–0.033	0.082	3.12	**1.43**

[a] SF_2, SF_4, SF_6, survival fraction at 2, 4 and 6 Gy, respectively. Mean values ± SEM are shown. Significant differences between vehicle control and cells treated with NZ28 and/or NVP-AUY922 are indicated (*$p \le 0.05$, **$p \le 0.01$, ***$p \le 0.001$).
[b] α and β values were derived from the linear quadratic equation $SF = \exp[-\alpha \times D - \beta \times D^2]$.
[c] D_{50}, dose [Gy] to reduce survival fraction to 50%.
[d] SER, sensitizing enhancement ratio = D_{50} (irradiation)/D_{50} (irradiation and drug). A SER greater than 1.20 is indicative for radiosensitization (indicated in bold).

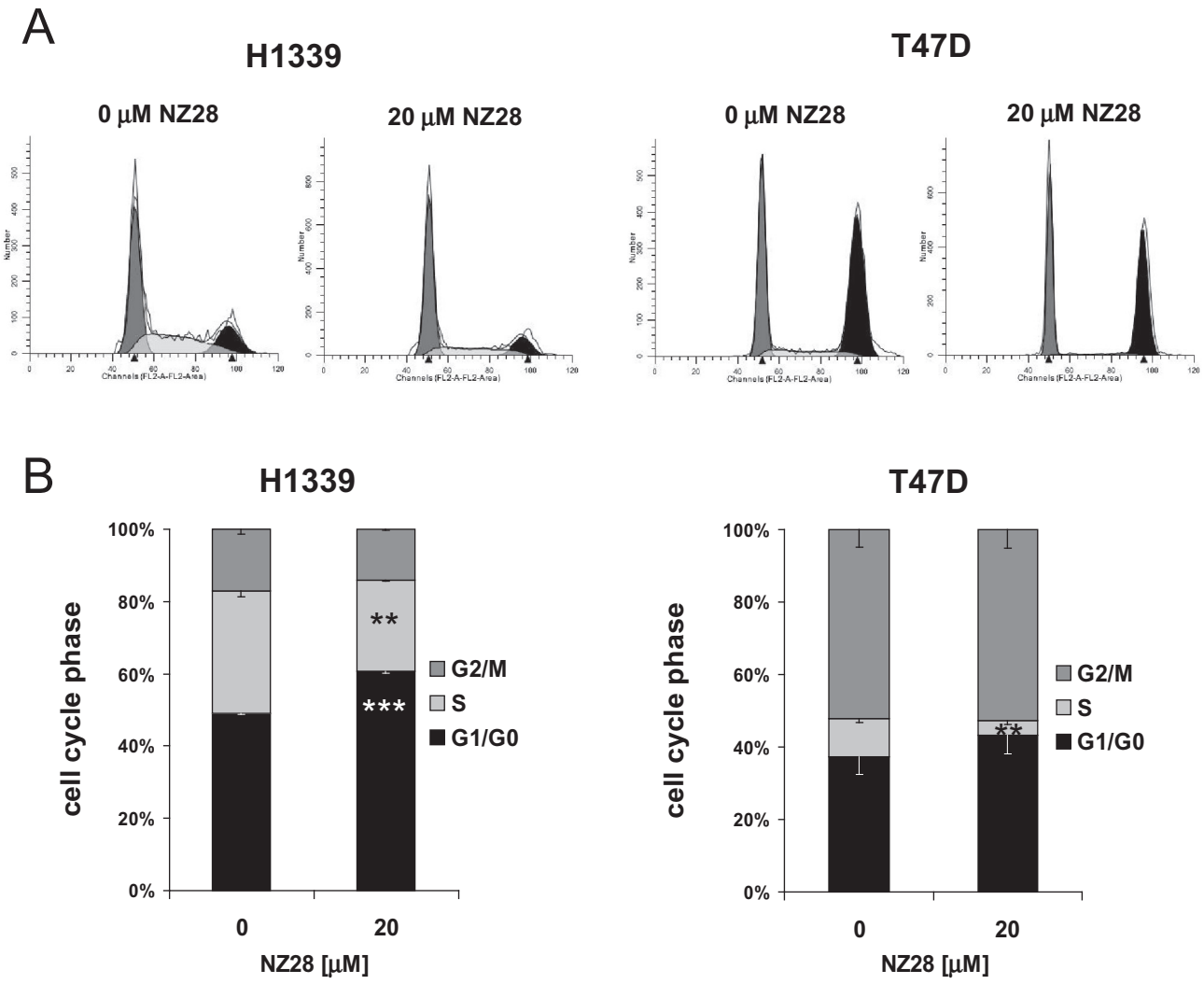

Fig. 4. NZ28 reduces the fraction of tumor cells in the radioresistant S-phase. The cell cycle distribution was determined after a 24 h treatment with 20 μM NZ28. DMSO (0.02%) treated cells served as control (0 μM NZ28). Representative histograms (A) and mean ± SEM (B) of 3 (H1339) and 5 (T47D) independent experiments are shown (*p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001).

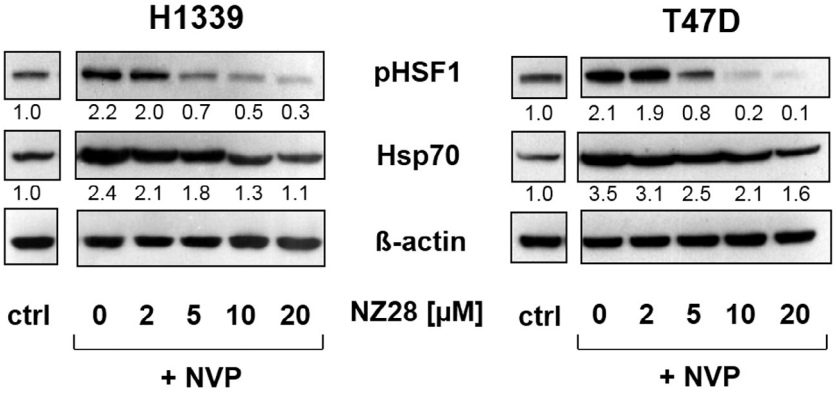

Fig. 5. NZ28 reduces NVP-AUY922-induced pHSF1 and Hsp70 levels. Representative HSF1 phospho S326 (pHSF1) and Hsp70 immunoblots. H1339 and T47D tumor cells were treated with 0, 2, 5, 10 and 20 μM NZ28 and 100 nM NVP-AUY922 (NVP) for 24 h. DMSO (0.2%) treated cells served as control (ctrl). A gap was included between control and treated samples because the lanes were not adjacent but samples were loaded on the same gel and the blot was exposed for the same period of time. The protein bands were quantified by densitometry using ImageJ. Numbers under the lanes represent the expression of pHSF1 or Hsp70 relative to β-actin. The value of control cells was set to 1 for each cell line.

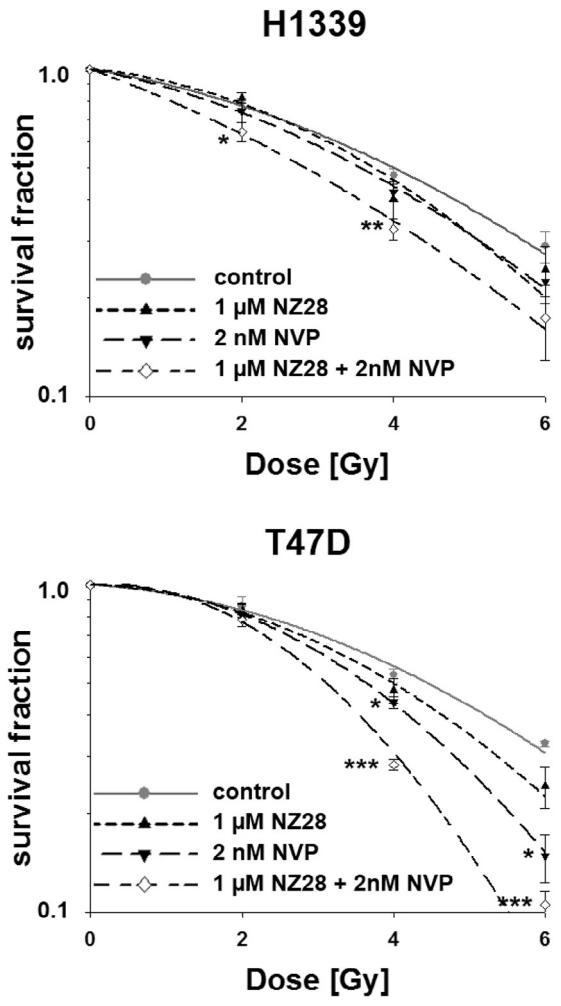

Fig. 6. Treatment with low concentrations of NZ28 and NVP-AUY922 potentiates radiosensitivity of tumor cells. One day after seeding H1339 and T47D cells were treated with 1 µM NZ28 and 2 nM NVP-AUY922 (NVP). 24 h after treatment cells were irradiated and immediately after irradiation fresh medium without NZ28/NVP-AUY922 was added. DMSO (0.01%) treated cells served as control. Colonies were fixed either 9 (H1339) or 16 (T47D) days after seeding. The survival fractions were calculated after normalization for cell kill by NZ28 and/or NVP-AUY922. Data are expressed as mean ± SEM of at least 3 independent experiments (*$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$).

significantly enhanced the radiosensitivity with SER values of 1.41 and 1.43 for H1339 and T47D cells, respectively (Fig. 6 and Table 1). The radiosensitization which was achieved by a combined treatment with low concentrations of NZ28 and NVP-AUY922 was comparable to that of a single treatment with NZ28 at 10–20-fold higher concentrations (Fig. 3B, Table 1).

Discussion

Hsp70 is abundantly overexpressed in a variety of human tumor cells and high Hsp70 levels are associated with poor clinical outcome since Hsp70 promotes tumor cell survival and contributes to radioresistance [10,12]. The group of M. Sherman demonstrated that the small molecule inhibitor NZ28 reduces both the stress-induced and basal Hsp70 expression levels in HER2-positive cancer cell lines [13,14]. In line with their data we show that NZ28 down-regulated basal and NVP-AUY922-induced Hsp70 expression in different lung and breast cancer cell lines. Moreover, we could show that NZ28 reduces the phosphorylation of HSF1 at serine 326 and thus inhibits HSF1 transcriptional activity as measured by a luciferase reporter assay. Concomitantly the Hsp70 mRNA levels were found to be reduced under non-stressed conditions. This suggests that NZ28 down-regulates Hsp70 expression via inhibition of HSF1. Zaarur et al. showed that NZ28 does not alter heat-induced Hsp70 mRNA levels and a weak inhibition of the HSF1 transcriptional activity [14]. Therefore, they propose a post-transcriptional reduction of the stress-induced Hsp70 expression by NZ28. This discrepancy might be explained by the fact that the HSF1 activation and Hsp70 expression is regulated differently by NZ28 under stressed and non-stressed conditions or by the use of different tumor cell lines, incubation times and NZ28 concentrations.

Previous studies demonstrated that knocking-down Hsp70 or HSF1 reduces the invasiveness of tumor cells [3,4,11]. Consistent with these findings, we could show that NZ28 decreases migration and invasion of different human tumor cell lines. Silencing HSF1 or Hsp70 by siRNA is assumed to increase the radiosensitivity of human tumor cell lines [9–12]. However, only few inhibitors targeting HSF1 and/or Hsp70 have been tested with respect to their radiosensitizing capacity and the published data have been controversially discussed. As an example, the small molecule Hsp70 inhibitor VER-155008 has been shown to increase the radiosensitivity of A549 lung cancer cells [27], whereas the HSF1 inhibitor KNK437 exerted an opposite effect in glioblastoma cells [28]. We could demonstrate that NZ28 increases the radiation response of different human lung and breast cancer cells. One possible mechanism might be a decrease of the radioresistant S-phase. In line with our data, the Hsp70 inhibitors, VER-155008 and pifithrin-µ have been found to exert identical effects on the cell cycle distribution [27,29,30].

Hsp90 inhibition is a promising strategy in cancer therapy. However, clinically applied Hsp90 inhibitors induce the synthesis of the cytoprotective chaperone Hsp70 by activation of HSF1. Therefore, it was not astonishing to find that inhibition of HSF1 and/or Hsp70 can sensitize tumor cells toward Hsp90 inhibitors [14,17–19,31–34]. In contrast, knowledge on the effects of HSF1/Hsp70 and Hsp90 inhibition on radiation therapy is limited [35]. Here, we could show that a combined treatment of tumor cells with low, non-toxic concentrations of NZ28 and NVP-AUY922 potentiates the radiosensitization of different tumor cells.

We have demonstrated that NZ28 reduces HSF1 activation and expression of cytoprotective Hsp70, inhibits migration and invasion, decreases the percentage of tumor cells in the radioresistant S-phase and increases their radiosensitivity. In combination with NVP-AUY922, 1/10th–1/20th of the concentration of NZ28 was sufficient to achieve a significant radiosensitization compared to the treatment with a single drug. Therefore, we assume that a simultaneous inhibition of Hsp90 and HSF1/Hsp70 combined with radiotherapy might provide a promising anti-cancer strategy.

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Conflict of interest

The authors state no conflict of interest.

Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.canlet.2015.02.033.

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