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Original Articles

Sensitizing tumor cells to radiation by targeting the heat shock response

Daniela Schilling [a,b], Annett Kühnel [a], Sarah Konrad [a], Fabian Tetzlaff [a], Christine Bayer [a,c], Julia Yaglom [d], Gabriele Multhoff [a,b,*]

[a] Department of Radiation Oncology, Technische Universität München, Munich, Germany
[b] Helmholtz Center Munich, German Research Center for Environmental Health, Institute of Biological and Medical Imaging, Munich, Germany
[c] German Cancer Consortium (DKTK) partner site Munich, German Cancer Research Center (DKFZ), Heidelberg, Germany
[d] Department of Biochemistry, Boston University School of Medicine, Boston, MA, USA

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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.

* Corresponding author. Department of Radiation Oncology, Technische Universität München, Ismaninger Str. 22, 81675 München, Germany. Tel.: +4989 41404514; fax: +4989 41404299.
E-mail address: gabriele.multhoff@lrz.tum.de (G. Multhoff).

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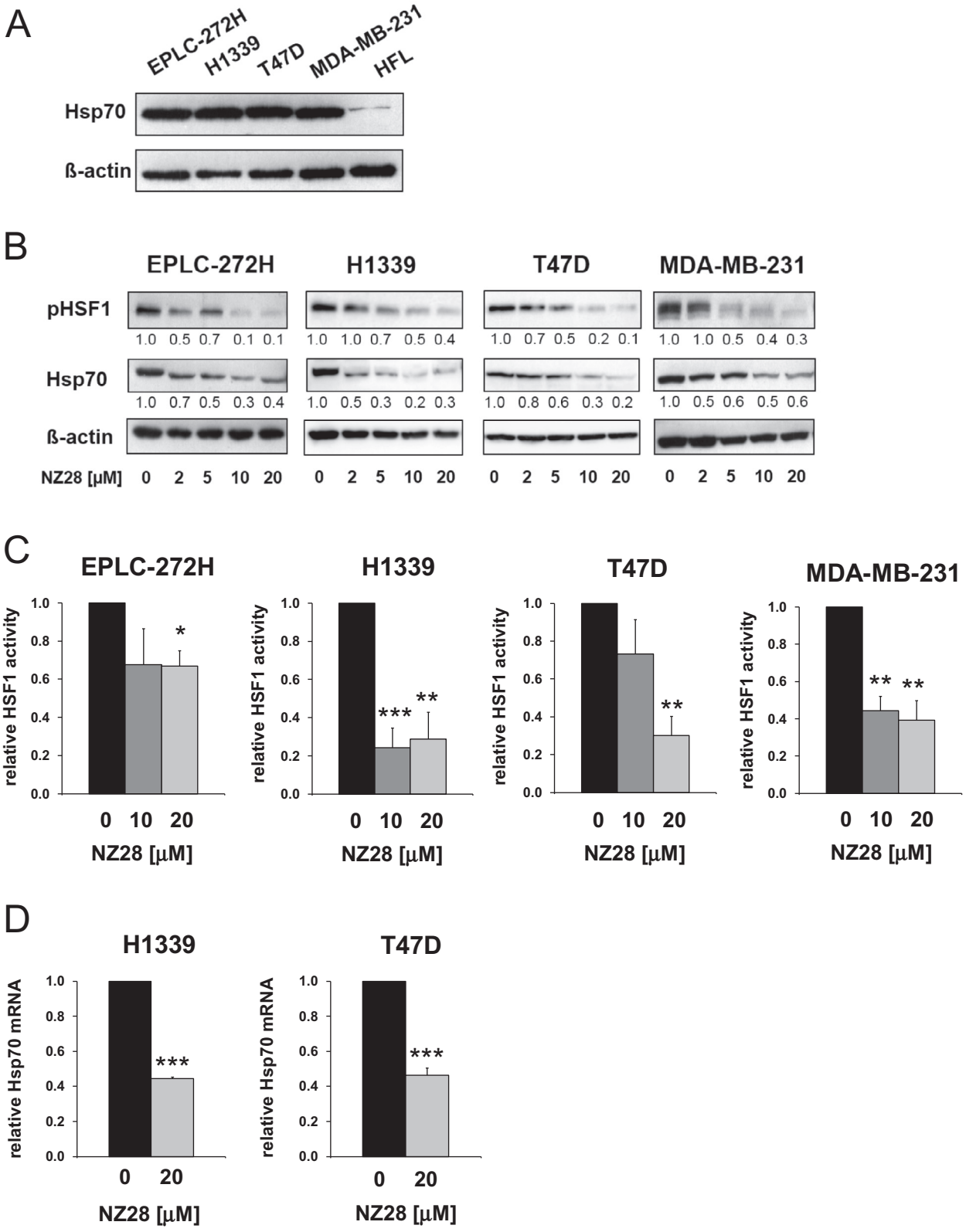

Fig. 1. NZ28 reduces HSF1 activity and Hsp70 expression in tumor cells. (A) Representative immunoblot showing the constitutive expression of Hsp70 in EPLC-272H, H1339, T47D and MDA-MB-231 tumor cells and human fetal lung fibroblasts (HFL). (B) Representative HSF1 phospho S326 (pHSF1) and Hsp70 immunoblots 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 ≤ 0.01, **p ≤ 0.01, ***p ≤ 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 ≤ 0.01).

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