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Reduced Levels of IGF-I Mediate Differential Protection of Normal and Cancer Cells in Response to Fasting and Improve Chemotherapeutic Index

Changhan Lee¹, Fernando M. Safdie¹, Lizzia Raffaghello³, Min Wei¹, Federica Madia¹, Edoardo Parrella¹, David Hwang², Pinchas Cohen², Giovanna Bianchi³, and Valter D. Longo¹

Abstract

Inhibitors of the insulin-like growth factor-I (IGF-I) receptor have been widely studied for their ability to enhance the killing of a variety of malignant cells, but whether IGF-I signaling differentially protects the host and cancer cells against chemotherapy is unknown. Starvation can protect mice, but not cancer cells, against high-dose chemotherapy [differential stress resistance (DSR)]. Here, we offer evidence that IGF-I reduction mediates part of the starvation-dependent DSR. A 72-hour fast in mice reduced circulating IGF-I by 70% and increased the level of the IGF-I inhibitor IGFBP-1 by 11-fold. LID mice, with a 70% to 80% reduction in circulating IGF-I levels, were protected against three of four chemotherapy drugs tested. Restoration of IGF-I was sufficient to reverse the protective effect of fasting. Sixty percent of melanoma-bearing LID mice treated with doxorubicin achieved long-term survival whereas all control mice died of either metastases or chemotherapy toxicity. Reducing IGF-I/IGF-I signaling protected primary glia, but not glioma cells, against cyclophosphamide and protected mouse embryonic fibroblasts against doxorubicin. Further, *S. cerevisiae* lacking homologs of IGF-I signaling proteins were protected against chemotherapy-dependent DNA damage in a manner that could be reversed by expressing a constitutively active form of Ras. We conclude that normal cells and mice can be protected against chemotherapy-dependent damage by reducing circulating IGF-I levels and by a mechanism that involves down-regulation of proto-oncogene signals. *Cancer Res*; 70(4); 1564–72. ©2010 AACR.

Introduction

Most chemotherapy agents cause considerable damage to normal cells, leading to toxicity, which is dose limiting, and causes both short- and long-term side effects in patients. Although drug development has reduced these side effects with a succession of selective antitumor agents, such as antibodies that target specific antigens in cancer or agents with a wider therapeutic index, toxicity continues to limit cancer treatment. Thus, interventions that reduce the undesired toxic side effects could increase the efficacy of many chemotherapy drugs. Chemoprotectants such as amifostine, glutathione, mesna, and dexrazoxane have been investigated and shown to provide drug-dependent protection to specific tis-

issues, but the use of these compounds has not been shown to increase disease-free or overall survival (1). Recently, we reported that short-term starvation (STS) protects normal but not, or much less, malignant cells, leading to improved survival [differential stress resistance (DSR); ref. 2]. Here we present evidence that reduced insulin-like growth factor I (IGF-I) is a major mediator of STS-dependent differential protection.

Biogerontologists have long known that calorie restriction (CR) and/or deficiencies in the pro-growth growth hormone (GH)/IGF-I axis increase stress resistance and life span in various model organisms (3). These beneficial effects can be explained, in part, by the active diversion of energy utilization in starved or IGF-I-deficient organisms. The finite energy source of an organism is finely balanced between growth and maintenance under normal conditions (4). However, under challenging conditions such as starvation, the energy is diverted from growth to maintenance, thereby enhancing protection and survival at the price of growth (5).

During starvation, several changes in the GH/IGF-I axis occur as a result of physiologic adaptation to the new environment. Generally, GH directly regulates the production of IGF-I, which is the major mediator of the growth effects of GH (6). In humans, IGF-I levels decrease dramatically in response to short-term starvation (36–120 hours) despite increased GH secretion (7, 8). Long-lived organisms that are deficient in IGF-I signaling have been shown to be resistant

Authors' Affiliations: ¹Andrus Gerontology Center, Department of Biological Sciences and Norris Cancer Center, University of Southern California; ²Pediatric Endocrinology, UCLA, Los Angeles, California; and ³Laboratory of Oncology, Giannina Gaslini Institute, Genova, Italy

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Corresponding Author: Valter D. Longo, Andrus Gerontology Center and Department of Biological Sciences, University of Southern California, 3715 McClintock Avenue, Los Angeles, CA 90089-0191. Phone: 213-740-6212; Fax: 213-821-5714; E-mail: vlongo@usc.edu.

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to multiple types of stress (9, 10). However, unlike normal cells, cancer cells are self-sufficient in growth signals and insensitive to growth inhibitory signals (11). Self-sufficiency in growth signals is often enabled by gain-of-function mutations in oncogenes [e.g., IGF-I receptor (IGF-1R) or its downstream effectors *Ras*, *PI3K*, etc.] that result in constitutive activation of proliferation pathways independently or partially independently of external growth factor level. Notably, in normal cells, the RAS/RAF/MAPK and the PTEN/PI3K/AKT pathways can be downregulated by CR or starvation (12), possibly by reducing IGF-I. On the other hand, insensitivity to growth inhibitory signals is due to loss-of-function mutations in tumor suppressor genes (e.g., *Rb*, *p53*, *PTEN*, etc.), enabling cancer cells to disregard antiproliferation signals (11, 13). Here we test the hypothesis that the reduction of circulating IGF-I and its signaling mediates the protection of normal cells and mice against chemotherapy toxicity, but not of oncogene-bearing cancer cells, which do not respond to reduced IGF-I.

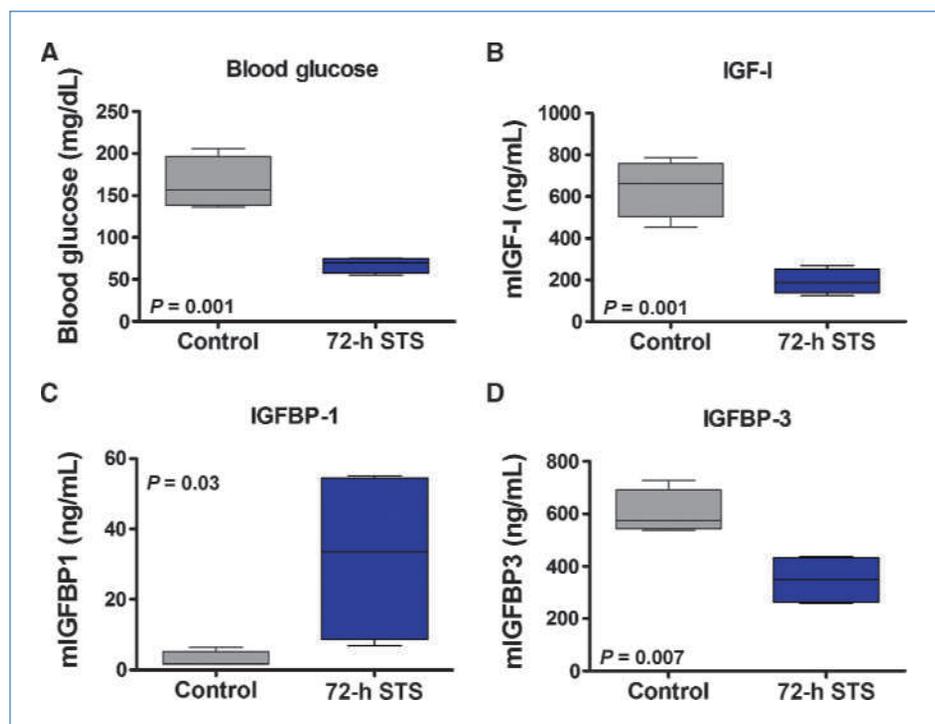
Materials and Methods

Stress resistance against chemotherapy treatments in LID mice. LID mice, 75 to 100 wk of age, were used to model human cancer treatment. Because liver is the major source of IGF-I production, mice with a conditional hepatic *Igf1* gene knockout have 80% reduced circulating IGF-I levels (14). Because albumin is expressed in the liver after 10 d of birth, resulting in liver *Igf1* gene deletion, LID mice do not experience early death, growth retardation, or developmental defects like the *Igf1* gene knockout (*Igf1*^{-/-}) mice (15). LID

and its control mice were given 100 mg/kg etoposide i.v. Cyclophosphamide was given at 500 mg/kg. Cyclophosphamide was dissolved in saline at 40 mg/mL and injected i.p. 5-Fluorouracil (5-FU; Sigma) was injected at 400 mg/kg i.p. Doxorubicin (DXR; Bedford Laboratories) was prepared at 5 mg/mL in saline and injected i.v. first at 20 mg/kg and 22 d later at 28 mg/kg. All drugs have been selected from different categories. All mice were monitored daily for weight loss and signs of pain and stress. Mice determined to be terminally moribund were euthanized by CO₂ narcosis and necropsy was done. Experiments were done in accordance with the Institutional Animal Care and Use Committee (University of Southern California, Los Angeles, CA) and NIH guidelines.

DSR against DXR in LID mice. To study DSR, mice were injected with highly metastatic melanoma cells. LID and its control mice of ages 75 to 100 wk were used. B16Fluc melanoma cells were a generous gift of Dr. Noah Craft at UCLA. B16Fluc cells are derivatives of B16 cells but produce light by stable transfection of the firefly luciferase gene driven by the cytomegalovirus promoter (16). Before injection, cells were washed and resuspended in sterile saline. Each mouse received 2×10^5 cells in 100 μ L of saline, followed by another 100 μ L of sterile saline to wash off remaining cells in the tails. Three days after tumor inoculation, the first DXR (Bedford Laboratories) injections were given at 16 mg/kg. Two weeks after the initial DXR administration, the second DXR injection was given at 12 mg/kg. Mice were observed daily for signs of stress or pain and body weight was recorded. Mice determined to be terminally moribund were sacrificed by CO₂ narcosis and necropsy was done. The heart was collected for further histologic examination.

Figure 1. The effect of 72-h fasting on glucose, IGF-I, and IGFBP-1/IGFBP-3 levels. Thirty-week-old CD-1 mice were fasted for 72 h and sacrificed. Blood was collected via cardiac puncture under anesthesia, and blood glucose (A) was measured immediately. Plasma IGF-I (B) and IGFBP-1/IGFBP-3 (C and D) levels were measured by a mouse-specific in-house ELISA. All *P* values were calculated by Student's *t* test except that for IGFBP-1, which was done by the Mann-Whitney *U* test.



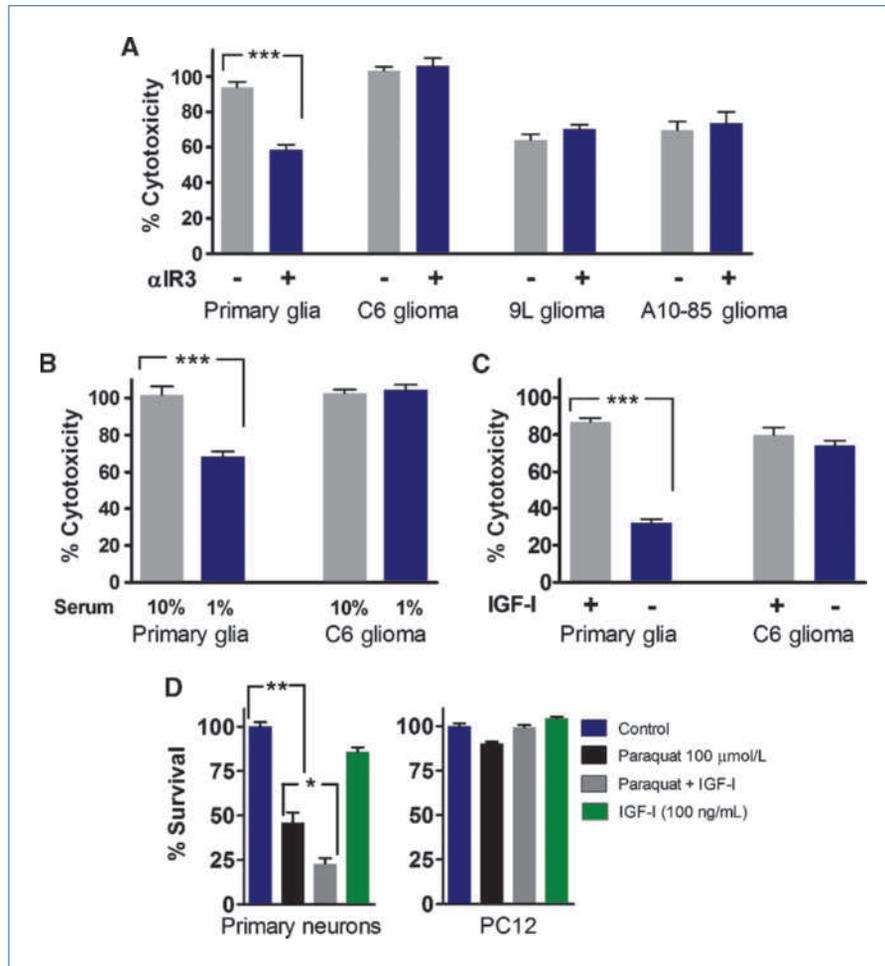


Figure 2. *In vitro* DSR to cyclophosphamide treatments by reducing IGF-I. Primary rat glial cells and rat glioma cell lines (C6, 9L, and A10-85) were tested. A, cells were preincubated in DMEM/F-12 with 1% serum and neutralizing anti-IGF-1R monoclonal antibody α -IR3 (1 μ g/mL) for 24 h (15 mg/mL; $n = 12$). B, cells were preincubated in medium with either 1% (STS) or 10% fetal bovine serum for 24 h (15 mg/mL; $n = 12$). C, cells were preincubated in medium with 1% serum with or without rhIGF-I (100 ng/mL) for 48 h (12 mg/mL; $n = 21$). D, effect of IGF-I on DSR against oxidative stress. Chemotherapy drugs such as etoposide, cyclophosphamide, 5-fluorouracil, and DXR have been shown to increase reactive oxygen species and cause oxidative stress. Primary neurons and PC12 pheochromocytoma cells were pretreated with IGF-I (100 ng/mL) for 30 min, followed by paraquat treatments for 24 h. Cytotoxicity (LDH assay) was determined following treatment. *, $P < 0.05$; **, $P < 0.005$; ***, $P < 0.0001$, Student's t test.

Results

Short-term starvation regulates components of the pro-growth GH/IGF-I axis. To investigate the role of the GH/IGF-I axis in the beneficial effects of STS on DSR, we measured the levels of circulating GH and IGF-I and its binding proteins, IGFBP-1 and IGFBP-3, in mice undergoing STS. CD-1 mice were fasted for 72 hours and blood was collected to measure glucose levels and plasma GH, IGF-I, IGFBP-1, and IGFBP-3 levels. After a 72-hour STS, mice had lost approximately 20% of body weight, glucose levels were reduced by 41%, GH levels were slightly increased, and IGF-I levels decreased by 70% (Fig. 1A and B; Supplementary Fig. S1A and B). The bioavailability of IGF-I, which can activate IGF-1Rs, is regulated by IGF binding proteins. In fasted mice, the level of IGFBP-1, which normally reduces IGF-I signaling, increased 11.4-fold (Fig. 1C). These results are in agreement with the reports showing that IGFBP-1 increases in response to fasting in humans and rats (17–19) and also that its over-expression in mice effectively retards growth by sequestering IGF-I (20). Furthermore, the 72-hour fast decreased IGFBP-3 levels by 42% (Fig. 1D), in agreement with reports on short-

term fasted humans and rats (19, 21). However, the mechanistic explanation for the decrease in IGFBP-3 is not clear.

To test if restoring the level of IGF-I during STS reverses the protection against chemotherapy toxicity, CD-1 mice underwent a 48-hour STS with IGF-I (200 μ g/kg) administration every 12 hours. The level of injected IGF-I was determined from prior serum IGF-I measurements of *ad libitum* fed mice. Following the STS/IGF-I treatments, mice were i.v. injected with 16 mg/kg DXR, a widely used chemotherapy drug acting as an intercalating agent and topoisomerase II inhibitor (22). Indeed, the restoration of IGF-I during STS abolished the protective effect of STS on DXR toxicity, resulting in a 100% versus 38% survival in the STS and STS/IGF-I groups, respectively (Supplementary Fig. S2).

Previously, we showed that primary glia, but not glioma cell lines, preincubated with low glucose (50 mg/dl compared with the normal 100 mg/dl) and low serum (1% fetal bovine serum) with the consequent reduction of several growth factors including IGF-I showed enhanced protection against the alkylating chemotherapy agent cyclophosphamide (2). The glucose levels of fasted mice were reduced to a similar level, along with a dramatic decrease in IGF-I levels (Fig. 1A

and B). Therefore, the reduction of IGF-I, a potent growth factor, mediates part of the effect of fasting on DSR.

Reduced IGF-I signaling protects primary glia, but not glioma cells, against high-dose cyclophosphamide. IGF-I-like signaling pathways are implicated in regulating life span and stress resistance in organisms ranging from the simple yeast to worms, flies, and mice (3, 23–25). To test the role of IGF-I signaling in DSR against chemotherapeutic drugs *in vitro*, we incubated normal and the equivalent cancer cell lines with an IGF-I receptor (IGF-1R) blocking antibody, low serum concentrations, or excess IGF-I before treatment with cyclophosphamide, a commonly used chemotherapy drug based on its DNA alkylating properties (26). Primary mixed rat glia (astrocytes + 5–10% microglia) and three different rat glioma cell lines (C6, A10-85, and 9L) were tested. All cells were grown to confluence to minimize differences in proliferation rate. Preincubation with an antagonistic IGF-1R antibody (α IR3) protected primary glia, but not the three glioma cell lines, against cyclophosphamide toxicity (Fig. 2A). Reduction of serum level from the standard 10% to 1%, with consequent reduction of growth factors including IGF-I, decreased the toxicity of 15 mg/mL cyclophosphamide to primary glia but not to C6 glioma cells (Fig. 2B). On the other hand, preincubation with 100 ng/mL IGF-I (in the low reference range for adult human serum; ref. 27) caused a 3-fold increase in the toxicity of cyclophosphamide to primary mixed glia but did not increase the toxicity of cyclophosphamide to C6 glioma cells (Fig. 2C). Similar results were obtained with primary neurons and neuron-like pheochromocytoma cells (PC12) treated with a combination of IGF-I and the oxidative stress agent paraquat (Fig. 2D). These results are consistent with our previous studies on fasting and DSR (2) and support the hypothesis that downregulation of IGF-I signaling can protect normal, but not cancer, cells against cytotoxic agents.

Effect of IGF-1R deletion or overexpression on stress resistance in mouse embryonic fibroblast cells. To begin to investigate the mechanism responsible for DSR, we treated mouse embryonic fibroblasts bearing an *Igf1r* deletion (R^- cells) or overexpressing IGF-1R (R^+ cells) with DXR (28). All cells were grown to confluence to minimize the difference in proliferation rate and were treated with DXR for 24 or 48 hours. After a 24-hour DXR treatment, R^- cells showed greater survival compared with R^+ cells. The effect was most pronounced at 25 μ mol/L where more than 80% of R^- cells were viable, whereas only 30% of R^+ cells were alive (Fig. 3A; $P < 0.0005$). Similar results were observed when cells were treated for 48 hours, with a 50% versus 12% survival rate for R^- and R^+ cells, respectively, at 25 μ mol/L (Fig. 3B; $P < 0.02$).

To further investigate how deficiency in IGF-I signaling protects against chemotoxicity, we measured DNA damage using the comet assay. DXR-induced DNA damage was significantly higher in R^+ cells compared with R^- cells, with a more than 3-fold difference as assessed by the comet assay (Fig. 3C and D; $P < 0.001$). These results support our hypothesis that reduced IGF-I signaling protects normal cells by reducing chemotherapy-dependent DNA damage (29). Notably, R^- cells have been shown to be resistant against transformation by the SV40 large T-antigen, which is remarkable con-

sidering that fibroblasts frequently transform in culture spontaneously (30).

The role of yeast homologs of downstream effectors of the IGF-1R in DSR. To investigate the mechanisms by which downregulation of the IGF-1R signaling protects against chemotoxicity and its effect on DNA damage, we turned to the simple model system *S. cerevisiae*. The rationale for using

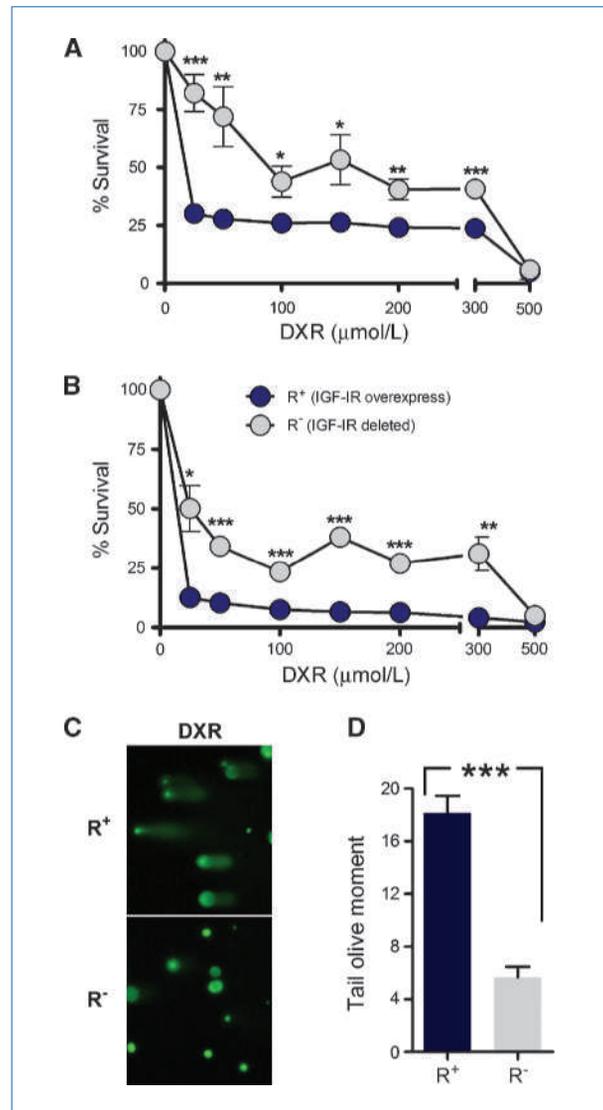


Figure 3. R^+ and R^- cells were grown to confluence and treated with DXR (0–500 μ mol/L) in DMEM/F-12 supplemented with 10% fetal bovine serum for 24 h (A) or 48 h (B). Viability was determined by the relative degree of MTT reduction compared with untreated. Points, mean; bars, SD. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$, R^+ versus R^- cells at same DXR concentration (Student's *t* test). C, comet assay. Cells overexpressing IGF-1R or with IGF-1R deficiency (R^+ and R^-) were treated with 50 μ mol/L DXR for 1 h. Significant DNA damage was observed in the DXR-treated R^+ cells, whereas R^- cells showed enhanced protection against DXR-induced DNA damage. D, tail olive moment analysis of the comet assay. ***, $P < 0.001$, R^+ DXR versus R^- DXR (Student's *t* test). Similar results were obtained from two independent experiments. A representative experiment is shown.

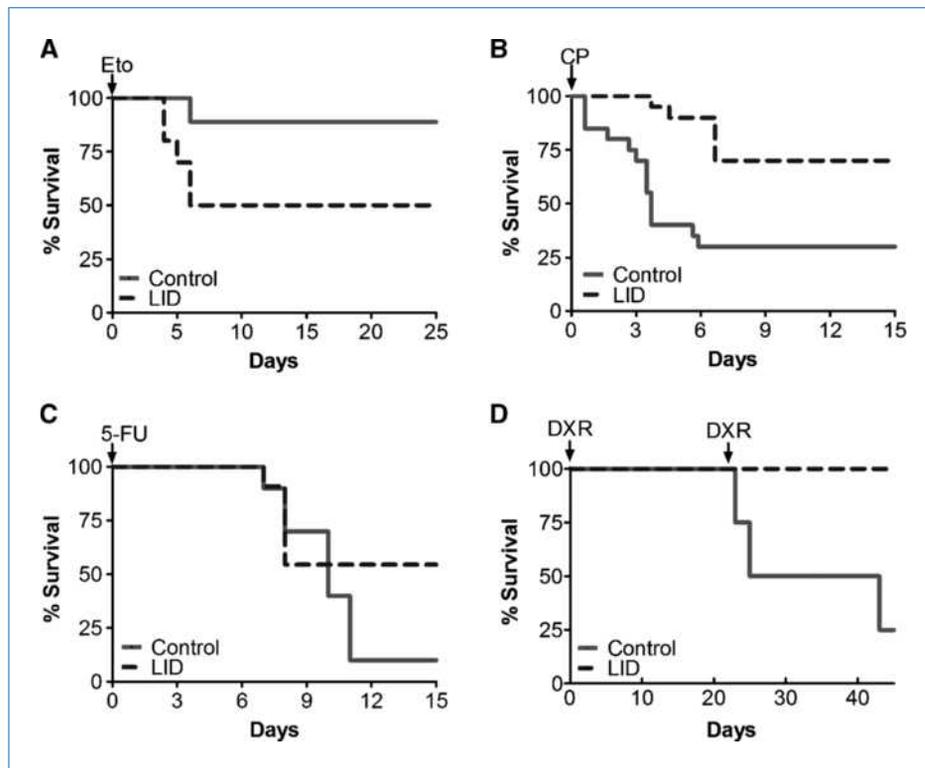


Figure 4. Stress resistance testing in LID mice with various high-dose chemotherapeutic drugs. LID and control mice received (A) a single injection of 100 mg/kg etoposide ($n = 10/\text{LID}$, $n = 9/\text{control}$, $P = 0.064$), (B) a single injection of 500 mg/kg cyclophosphamide (CP; $n = 20/\text{group}$, $P = 0.001$), (C) a single injection of 400 mg/kg 5-fluorouracil ($n = 11/\text{LID}$, $n = 10/\text{control}$, $P = 0.148$), or (D) two injections of DXR. The first injection of 20 mg/kg was given on day 0, and the second injection of 28 mg/kg was given on day 22 ($n = 5/\text{LID}$, $n = 4/\text{control}$, $P = 0.022$). Toxicity evaluated by percent survival is shown. P values by Peto's log-rank test.

yeast is based on the role of Ras2 and Sch9, homologs of the mammalian Ras and S6K, respectively, in modulating cellular defense against oxidative stress, DNA alkylation, and thermal stress, which was demonstrated in our previous studies (2, 31, 32), and on the central signaling role of homologs of *SCH9* and *RAS2* downstream of IGF-1R (Supplementary Fig. S3A). We tested the effect of the deletion of *RAS2* and *SCH9* on the resistance against DXR. To further investigate DSR, we also studied cells transformed with a gene expressing a constitutively active *RAS2* (*RAS2^{val19}*) that models human oncogenic Ras mutations. The deletion of *SCH9* (*sch9Δ*) or both *SCH9* and *RAS2* (*sch9Δ ras2Δ*) provided remarkable protection against DXR compared with its wild-type (WT) strains (Supplementary Fig. S3B). However, analogous to what we observed in mammalian cells, the expression of the oncogene-like *RAS2^{val19}* reversed the protection provided by *RAS2* and *SCH9* deficiency. After 48 hours of DXR treatment, 50% of WT and *RAS2^{val19}*-expressing cells survived, whereas 70% of *sch9Δ* and more than 90% of *sch9Δ ras2Δ* survived (Supplementary Fig. S3B). The protection was more pronounced after 72 hours of DXR treatment, where *sch9Δ ras2Δ* and *sch9Δ* were highly protected (88% and 70% survival, respectively), but the protection was reversed by the expression of *RAS2^{val19}* (*sch9Δ RAS2^{val19}*; 27% survival). To study the molecular mechanisms of differential resistance to DXR, we monitored DNA mutation frequency, measured as mutations in the *CAN1* gene (Can^r colonies/ 10^6 cells; ref. 33). DXR treatments increased mutation frequency in all strains. In agreement with the survival analysis, *sch9Δ* and *sch9Δ ras2Δ* exhibited the lowest mutation fre-

quency, whereas *RAS2^{val19}* expression increased mutation frequency (Supplementary Fig. S3C). The expression of *RAS2^{val19}* in *sch9Δ* (*sch9Δ RAS2^{val19}*) completely reversed the protection provided by the *Sch9* deficiency, resulting in a 3-fold increase in mutation frequency (Supplementary Fig. S3B and C). These data suggest that lowered Ras2 and Sch9 signaling has a beneficial effect that could be, at least in part, due to the enhanced protection against DNA damage in the cell, which can be reversed by the expression of oncogenes.

Octreotide sensitizes *NXS2* neuroblastoma cells but does not protect mice against high-dose etoposide. Because reduction of IGF-I provided differential chemotherapy protection in mammalian cell culture, we tested if pharmacologic manipulation of the GH/IGF-I axis could induce DSR *in vivo*. The somatostatin analogue octreotide is used in clinics to reduce GH secretion and IGF-I production primarily in acromegaly patients. In addition, octreotide was selected because somatostatin increases in response to fasting (34). Interestingly, octreotide and other somatostatin analogues have been shown to have therapeutic effects in a number of cancers (35) through two distinct effects: direct actions on tumors mediated by somatostatin receptors (36, 37) and indirect effects through inhibition of GH release and the lowering of IGF-I (36–38). In a previous report, we showed that STS provides DSR against high-dose etoposide (2), a widely used chemotherapy drug that inhibits topoisomerase II (39). Here, we tested if inhibiting the GH/IGF-I axis with octreotide could increase the protection against etoposide. We selected a particularly aggressive tumor line (*NXS2*) that models neuroblastoma (40). I.v. injection

of NXS2 cells results in a consistent formation of metastasis in multiple organs including the liver, kidneys, adrenal gland, and ovaries (40). A single injection of high-dose etoposide (80 mg/kg) extended the life span of tumor-bearing mice, which otherwise would have succumbed to metastasis within 40 days. In our previous study, STS caused a remarkable reduction in acute chemotoxicity-related deaths, but it also provided partial protection to the cancer cells (2). Our present results indicate that octreotide is not sufficient to protect the animals against chemotherapy, but its combination with STS sensitizes the NXS2 cancer cells to etoposide (Supplementary Fig. S4A and B; Supplementary Fig. S4C, gr. 4 versus gr. 7; $P < 0.01$). However, octreotide had a minimal effect on lowering IGF-I levels in mice (Supplementary Fig. S4D), which could explain its inability to

protect the animal. It is possible that homeostatic mechanisms counteract the effect of somatostatin and lead to tachyphylaxis to octreotide treatment, thus failing to reduce IGF-I levels significantly.

To test if octreotide exerted its sensitizing effect on NXS2 cells directly or indirectly, we treated NXS2 cells with octreotide and etoposide *in vitro* (Supplementary Fig. S4E). Octreotide did not alter the toxicity of etoposide to NXS2 cells in cell culture, suggesting that the sensitizing effect of octreotide in mice is indirect. Together, this implies that octreotide alone does not provide starvation-like host protection, but it may reverse the partial protection provided by STS to cancer cells by sensitizing them. Further studies are necessary to investigate the possibility that octreotide may sensitize other tumors against chemotherapy.

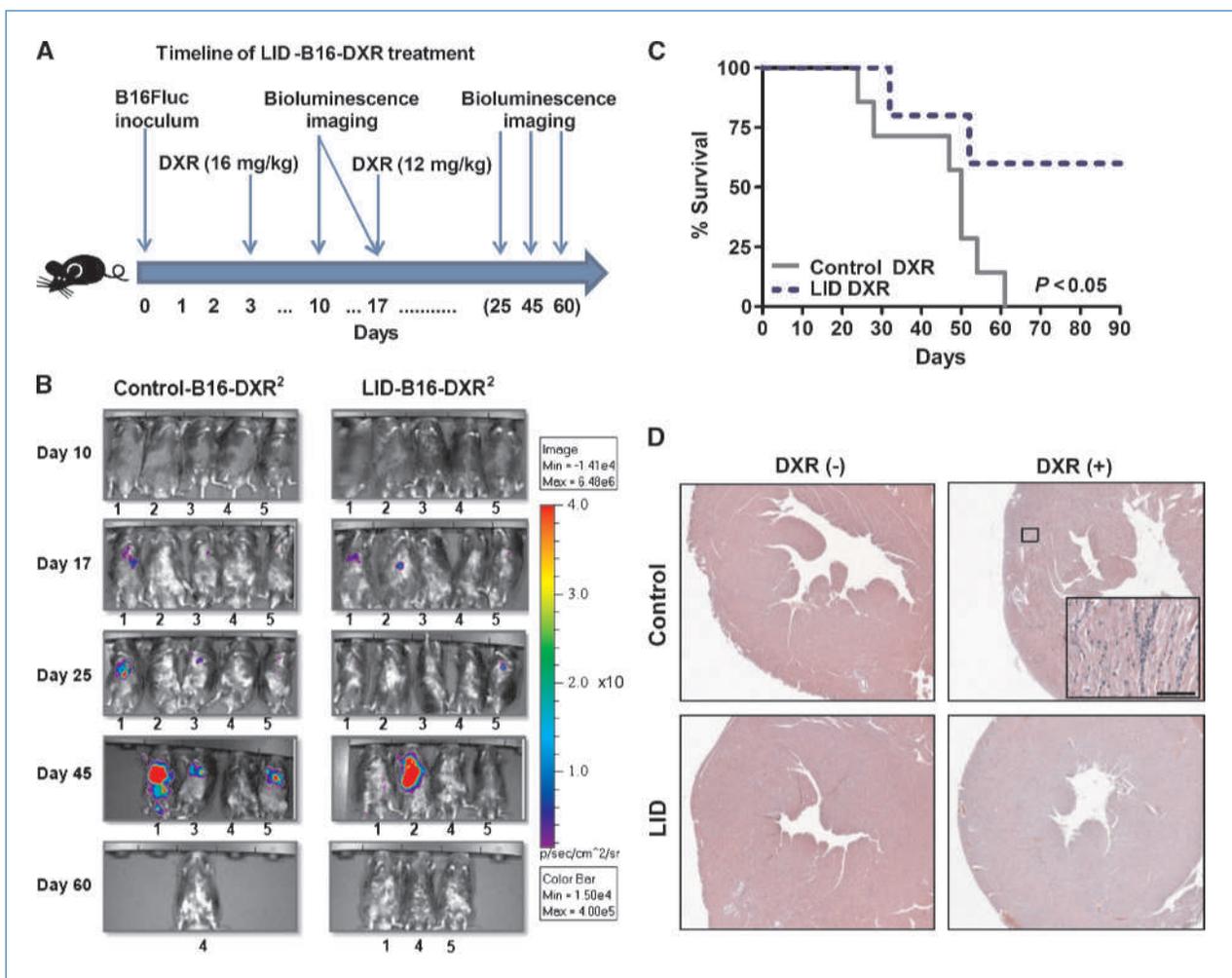


Figure 5. DSR against two cycles of high-dose DXR in melanoma-bearing LID mice. A, timeline of experimental procedures ($n = 4$ /LID-B16, $n = 5$ /LID-B16-DXR, $n = 8$ /control-B16, $n = 7$ /control-B16-DXR). B, bioluminescence imaging of B16Fluc melanoma-bearing LID and control mice treated with two cycles of high-dose DXR. Five mice were randomly selected and followed throughout the experiment to monitor tumor progression or regression. C, survival rate comparison between B16Fluc melanoma-bearing LID and control mice treated with two cycles of high-dose DXR ($P < 0.05$). D, DXR-induced cardiomyopathy in control and LID mice. Heart failure is a major outcome of acute DXR toxicity. Histologic slides of the heart from DXR-treated control mice showed loss of myofibrils and infiltration of immune cells, whereas DXR-dependent cardiac myopathy was not observed in LID mice. H&E staining. A representative slide is shown. Bar, 100 μ m.

Enhanced stress resistance in LID mice against high-dose chemotherapy. Mice with genetic disruptions in the IGF-1R or its downstream effectors are more resistant to oxidative stress (9, 41). To determine whether reducing IGF-I signaling protects from chemotoxicity, we tested a transgenic mouse model with a conditional liver *Igf1* gene deletion (LID; ref. 15) resulting in a 70% to 80% postnatal reduction of circulating IGF-I (14), which is similar to that of a 72-hour fasted mouse (Fig. 1B). The LID mice provide a model for investigating the mechanistic relationship between IGF-I and fasting in chemotherapy resistance (42). A single administration of high-dose etoposide led to a 50% versus 88% survival rate, respectively, in the LID and control mice (Fig. 4A; Supplementary Fig. S5A). Based on our *in vitro* results, we tested cyclophosphamide in LID mice. LID mice treated with 500 mg/kg cyclophosphamide showed significantly higher resistance, with a 70% versus 30% survival rate for LID and control mice, respectively (Fig. 4B). Furthermore, whereas LID mice only lost an average of 10% of their weight, control mice lost 20% (Supplementary Fig. S5B). The surviving LID mice also did not show signs of toxicity (Supplementary Fig. S6). To determine the range of protection by reduced IGF-I, we tested two additional drugs, 5-FU and DXR, which represent different classes of chemotherapy drugs. 5-FU is an antimetabolite (43). Survival after a treatment with high-dose 5-FU was improved, with a 55% versus 10% survival rate in LID and controls, respectively, although the difference was not statistically significant (Fig. 4C). Similar but more pronounced effects were observed with DXR. Unlike etoposide and other drugs that can cause irreversible damage to the tail vein of rodents after a single high-dose injection, DXR can be injected for up to two to three cycles (data not shown). To test the effect of multiple cycles of chemotherapy, we challenged LID mice with two cycles of high-dose DXR. The first DXR injection (20 mg/kg) did not result in any toxicity-related deaths, but led to considerable weight loss in all mice (Fig. 4D; Supplementary Fig. S5C). Weight loss was more evident in LID mice during the first 5 days following DXR injection, but unlike the controls that continued to lose weight and showed signs of toxicity, LID mice regained their weight during the following 3 weeks. The second DXR injection (28 mg/kg) caused a considerable amount of DXR-related deaths in the control (25% survival) but not in the LID mice (100% survival; Fig. 4D).

DSR in melanoma-bearing LID mice against high-dose DXR. Next, we tested DSR *in vivo* by monitoring LID mice inoculated with a highly aggressive melanoma cell line (B16Fluc) that metastasizes primarily to the lungs (16) and treating them with DXR. B16Fluc is a luminescent derivative of the B16 mouse melanoma cell line. Therefore, tumor progression and regression can be visualized and quantified *in vivo* using bioluminescence imaging technology (16). LID and its control mice were i.v. injected with B16Fluc (2×10^5 cells per mouse) melanoma cells and treated with two cycles of high-dose DXR (Fig. 5A). Although IGF-I plays a major role in tumor growth and metastasis (44), both LID and its control mice started to succumb to metastasis as early as 25 days following cancer inoculation. The two cycles of high-dose DXR extended survival time by delaying metastasis in all mice

(Fig. 5C). Forty-three percent of control mice died with signs of DXR-induced cardiac myopathy, whereas none of the LID mice died from DXR toxicity (Fig. 5D; Supplementary Fig. S7A). In addition, LID mice showed a slight advantage in weight maintenance (Supplementary Fig. S8). Ninety days after cancer inoculation, all control mice that received chemotherapy had died from either cancer metastases or DXR toxicity, but 60% of LID mice that received two cycles of high-dose DXR treatment were cancer-free with no apparent toxic side effects (Fig. 5B and C; Supplementary Fig. S7). All the LID mice deaths were caused by cancer metastases. The progression of cancer between control and LID mice was similar after DXR treatments (Supplementary Fig. S7B), suggesting that the reduction of circulating IGF-I protects the host cells, but not cancer cells, against high-dose chemotherapy.

Discussion

In a previous report, we described a STS-based DSR method to protect the host cells, but not cancer cells, against high-dose chemotherapy. The basis for this seems to be the existence of a nondividing state, which normal cells enter in response to starvation for the purpose of investing the remaining energy resources in cellular protection against various insults. Here, we show that a major reduction in circulating IGF-I can protect the host cells, but not cancer cells, against chemotherapy. Low levels of IGF-I can reduce intracellular mitogenic signaling pathways, including those regulated by Ras and Akt, two of the major pathways downstream of the IGF-1R. We believe that the reduction of mitogenic stimuli may protect normal cells in part by inducing cell cycle arrest (45–47) and in part by shifting the energy toward protection and repair genes such as FOXO, SODs, and DNA repair genes (2, 10, 29, 46, 48). In yeast, we have previously shown that protection can be increased in nondividing cells by up to 1,000-fold, suggesting that a major component of the protective mechanisms is independent of the switch from a dividing to a nondividing state, at least in this simple organism (2). This is also in agreement with the effect of IGF-1R overexpression in sensitizing fibroblasts grown to confluence to DXR (Fig. 3). On the other hand, cancer cells are self-sufficient in growth signals, less or not responsive to physiologic antigrowth signals, and, in many cases, do not undergo cell cycle arrest due to checkpoint dysregulation (11, 29, 47). In fact, it has been shown that pretreatment with nontoxic doses of cell cycle arresting drugs (e.g., DXR) or growth factor inhibitors (inhibitors of MAP/ERK kinase or epidermal growth factor receptor) permits selective killing of checkpoint-deficient cells (49–51).

In support of our hypothesis, our yeast experiments show that deletion of the homologs of *RAS* and/or *SCH9* (AKT/S6K) promotes protection against DXR, but expression of the oncogenic *RAS2^{Val19}* reverses this cellular and DNA protection independently of cell division. These results raise the possibility that oncogenic mutations that activate pathways such as Ras, AKT, or PKA may reverse the protective effect of reduced IGF-I signaling in malignant cells, thus allowing differential protection of host and various cancers. Notably,

inhibition of the pathway downstream of oncogenic mutations could have either a positive or a negative effect on the protection of cancer cells. Preclinical studies show that IGF-1R targeting strategies can be effective in the treatment of multiple myelomas and prostate, breast, and colon cancers in addition to other cancers (38, 52). The antitumor effect seen with such agents is thought to be dependent on apoptosis resulting from IGF-1R inactivation (52). However, it must be noted that IGF-1R blockade could also trigger apoptosis in normal cells and may not protect against high-dose chemotherapy by interfering with the growth/recovery of certain types of cells (e.g., bone marrow cells). As observed with our LID mice, reduced IGF-I, unlike IGF-1R blockade, does not cause cancer cell death but can selectively enhance the resistance of normal cells against chemotoxicity and may sensitize cancer cells to chemotherapy. This is in agreement with the normal development of prostatic carcinoma in the LID-TRAMP model (14). Based on our results from etoposide-treated LID mice, strategies that reduce circulating IGF-I may also increase the toxicity of certain chemotherapy drugs. Therefore, the compatibility between each drug and IGF-I reduction/blockade therapy should be carefully tested in preclinical studies before being considered as a candidate. Although it seems to be central, IGF-I may represent simply one of a number of growth factors that can activate Ras, Akt, etc., in normal cells and promote cell death in cancer cells and, therefore, only one of the factors that can be downregulated to provide DSR (47).

In summary, our studies in mice indicate that major reduction in circulating IGF-I and in intracellular IGF-I signaling

enhances the resistance of the host cells, but not of cancer cells, against chemotherapy, thus providing the foundation for a method to augment cancer treatment without the need to fast. However, the combination of fasting and IGF-I reduction could result in an even more pronounced effect.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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