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Targeting cancer with a lupus autoantibody[#]

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Abstract

Systemic lupus erythematosus (SLE) is distinct among autoimmune diseases due to its association with circulating autoantibodies reactive against host DNA. The precise role that anti-DNA antibodies play in SLE pathophysiology remains to be elucidated, and potential applications of lupus autoantibodies in cancer therapy have not previously been explored. Here we report the unexpected finding that a cell-penetrating lupus autoantibody, 3E10, has potential as a targeted therapy for DNA-repair deficient malignancies. We find that 3E10 preferentially binds DNA single-strand tails, inhibits key steps in DNA single-strand and double-strand break repair, and sensitizes cultured tumor cells and human tumor xenografts to DNA-damaging therapy, including doxorubicin and radiation. Moreover, we demonstrate that 3E10 alone is synthetically lethal to

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BRCA2-deficient human cancer cells and selectively sensitizes such cells to low dose doxorubicin. Our results establish an approach to cancer therapy that we expect will be particularly applicable to BRCA2-related malignancies such as breast, ovarian, and prostate cancers. In addition, our findings raise the possibility that lupus autoantibodies may be partly responsible for the intrinsic deficiencies in DNA repair and the unexpectedly low rates of breast, ovarian, and prostate cancers observed in SLE patients. In summary, this study provides the basis for the potential use of a lupus anti-DNA antibody in cancer therapy and identifies lupus autoantibodies as a potentially rich source of therapeutic agents.

Introduction

Aberrant production of autoantibodies reactive against host DNA is a hallmark of systemic lupus erythematosus (SLE), and a subset of lupus autoantibodies penetrate living cells and nuclei (1). Many lupus autoantibodies are cytotoxic, but an unusual cell-penetrating lupus anti-DNA antibody that is not harmful to cells or tissues, 3E10, was isolated from a mouse model of SLE (2, 3). 3E10 is distinguished from most lupus autoantibodies by its benign toxicity profile and by its ability to penetrate cells via a mechanism that is independent of its constant domains. Specifically, the 3E10 single chain variable fragment (3E10 scFv, Fig. S1) penetrates cells and nuclei (Fig. 1A) through an equilibrative nucleoside transporter (ENT2) that is ubiquitous on human cells, including malignant cells (4, 5). 3E10 was shown to be safe in a human Phase I SLE vaccine clinical trial (6), but, due to the unexpected ability of 3E10 to penetrate into cell nuclei, its use as a vaccine was not further pursued. Instead, 3E10 was developed as a molecular delivery vehicle, and both 3E10 and its single chain variable fragment have proven effective in delivering cargo proteins to cell nuclei in culture and in animals (7–9). A 3E10 scFv-Hsp70 fusion protein (Fv-Hsp70), for example, was shown to penetrate neurons and increase their survival in the presence of oxidative stress in culture and to decrease cerebral infarct volumes *in vivo* (7, 8). In investigating the potential use of 3E10 scFv to deliver molecules to cells that modulate sensitivity to ionizing radiation (IR), we made the unexpected discovery that 3E10 alone has potential as a targeted therapy for malignancies with deficiencies in DNA repair.

Results

3E10 scFv sensitizes cancer cells to DNA-damaging therapy

We initially sought to test the ability of the 3E10 scFv-Hsp70 fusion protein to protect cells against IR, and this led us to discover that 3E10 scFv, by itself, enhances cellular sensitivity to DNA-damaging therapy. 3E10 scFv-Hsp70 provided a degree of radioprotection to MCF-7 human breast cancer cells, but 3E10 scFv by itself unexpectedly sensitized the cells to IR (Fig. 1B). 3E10 scFv has previously been shown capable of penetrating into the CNS *in vivo* (8), and, since IR is a key component of the standard of care for CNS malignancies such as glioblastoma multiforme, we next examined the impact of 3E10 scFv on the radiosensitivity of human glioma cell lines. Similar to the MCF-7 cells, U251 human glioma cells were more sensitive to IR in the presence of 3E10 scFv (Fig. S2). 3E10 scFv also sensitized U87 human glioma cells to doxorubicin, but not to paclitaxel (Figs. 1C and S3). In the absence of IR or doxorubicin, 3E10 scFv was not toxic to the MCF-7, U251, or U87 cells. Since both IR and doxorubicin induce DNA strand breaks (10) while paclitaxel interferes with microtubule function but does not directly damage DNA (11), these results suggest that the antibody selectively potentiates cell killing by DNA-damaging therapies.

3E10 sensitizes human glioma xenograft tumors to DNA-damaging therapy

The impact of 3E10 on cellular sensitivity to DNA-damaging therapy was also observed *in vivo*. Treatment of U87 human glioma xenograft tumors in nude mice with a combination of the full 3E10 antibody (administered by intraperitoneal injection) and 8 Gy IR suppressed tumor growth to a significantly greater degree than treatment with 8 Gy alone (Figs. 1D and 1E), with tumor tripling time 9.5 ± 0.5 (SEM) days in tumors treated with 8 Gy as compared to 13.7 ± 1.8 (SEM) days in tumors treated with 8 Gy + 3E10 ($p=0.04$). 3E10 alone, however, had no impact on U87 tumors relative to control buffer alone, with a tumor tripling time for control tumors of 6.8 ± 0.7 (SEM) days versus 6.5 ± 0.3 (SEM) days in tumors treated with 3E10 alone ($p=0.67$). In addition, 3E10 significantly sensitized U87 xenografts to low dose doxorubicin (Fig. S4). These data demonstrate sensitization of human glioma xenografts to DNA-damaging therapy by 3E10 *in vivo*. The full 3E10 antibody was used for these *in vivo* studies due to its expected longer half-life in circulation compared to the variable fragment, and these data therefore also confirm that the full 3E10 has sensitizing activity, similar to its single chain variable fragment.

3E10 preferentially binds DNA single-strand tails and inhibits DNA repair

As a potential mechanism for the sensitization of tumors to DNA-damaging therapy by 3E10, we hypothesized that DNA-binding by the antibody might inhibit DNA repair. The binding affinity of 3E10 for DNA substrates in several conformations was determined (Figs. 2A, S5, 2B); 3E10 bound substrates with a free single-strand tail with a K_d of $0.2 \mu\text{M}$ versus $0.4 \mu\text{M}$ for substrates without a single-strand tail. The association of 3E10 with single-stranded DNA was also directly observed under electron microscopy (EM) (Fig. S6). The observed preferential binding of 3E10 to single-strand tails is consistent with our previous finding that single-stranded poly-dT is a potent competitive inhibitor of the binding of 3E10 to duplex DNA (2), and these results raise the possibility that 3E10 interferes with DNA repair by preferentially binding DNA repair intermediates, which typically consist of duplex DNA with single-stranded tails. To test this hypothesis, the effect of 3E10 on the single-strand break/base excision repair (BER) pathway was examined. In BER, a damaged base is excised by a glycosylase followed by cleavage of the phosphodiester backbone by an endonuclease to yield a substrate with a single-strand break (product n). The dRP lyase activity of DNA polymerase β removes the dRP group, and its polymerase activity inserts the missing nucleotide and restores correct base pairing (product $n+1$). This is followed by ligation of the residual strand break by ligase to restore the integrity of the phosphodiester backbone, leading to conversion of the $n+1$ product into the full-length product in duplex conformation. The efficiency of BER may therefore be determined by tracking the amounts of the n and $n+1$ species over time as quantified relative to the percentage of total substrate and product DNA. The impact of 3E10 on BER was measured *in vitro* by examining the repair of a uracil in a synthetic radiolabeled duplex DNA substrate incubated with requisite repair enzymes including uracil DNA glycosylase, AP endonuclease, DNA polymerase β , and ligase in the presence of control buffer or $20 \mu\text{M}$ 3E10. The repair reaction was stopped at specific time points, and the n , $n+1$, and duplex reaction products were quantified by gel electrophoresis and autoradiography as previously described (12). 3E10 reduced the efficiency of BER, whereas a control anti-tubulin antibody had no impact on the pathway (Figs. 2C, 2D, and S7).

3E10 also interfered with key steps in homology-directed repair (HDR), which is one of the main pathways for the repair of DNA double-strand breaks (DSBs) (13–15). Specifically, 3E10 inhibited RAD51-mediated strand exchange in a dose-dependent manner, while a control anti-His₆ antibody had no effect on strand exchange (Figs. 3A–C, S8). In addition, direct visualization by EM revealed that RAD51 nucleofilament assembly was impaired by 3E10 (Fig. S9). We therefore predicted that the antibody would interfere with the repair of

DNA DSBs in cells exposed to DNA-damaging agents. As expected, resolution of DNA DSBs was delayed in U251 human glioma cells as measured 24 hours after irradiation in the presence of 3E10 scFv (Figs. 3D and 3E). Finally, we directly analyzed the impact of 3E10 on HDR in cells using the Direct Repeat-Green Fluorescent Protein (DR-GFP) chromosome-based fluorescent assay that reports recombination between direct repeat sequences after creation of a site-specific DSB by transfection of the meganuclease, I-SceI (16). In this assay, U2OS cells stably transfected with the DR-GFP recombination substrate produce GFP only after successful HDR of the induced DSB. As expected, less than 0.25% of the mock-transfected cells exhibited GFP expression. In cells transfected with I-SceI, $5.2 \pm 0.4\%$ (SEM) of control cells were GFP positive compared to $2.6 \pm 0.2\%$ (SEM) in cells treated with 3E10 ($p=0.001$), which provides a direct demonstration of an approximately 50% reduction in HDR efficiency mediated by 3E10 in cells (Fig. S10). Taken together, the above results demonstrate that 3E10 impairs both single-strand and double-strand break repair, providing a mechanism to explain the sensitization of cells and tumors to DNA-damaging therapy by 3E10.

3E10 is synthetically lethal to BRCA2-deficient cancer cells

Cancer cells harboring deficiencies in HDR due to BRCA2 mutations (17) are highly vulnerable to killing by inhibition of single-strand break repair (12, 18, 19), a phenomenon termed synthetic lethality (12, 18–20). In addition, BRCA2-deficient cancer cells can be killed by further inhibition of HDR (20). Based on our observation that 3E10 inhibits both single-strand break repair and HDR, we hypothesized that 3E10 would be synthetically lethal to BRCA2-deficient cancer cells. Consistent with this hypothesis, when tested on a matched pair of BRCA2-deficient (PEO1) and BRCA2-proficient (PEO1 C4-2 and PEO4) human ovarian cancer cells (21), both 3E10 and 3E10 scFv, by themselves, were toxic to the BRCA2-deficient cancer cells but not the BRCA2-proficient cells (Figs. 4A, S11). Treatment with 3E10 scFv alone also reduced the surviving fraction of BRCA2-deficient CAPAN1 human pancreatic cancer cells to 0.75 ± 0.03 (SEM) relative to control (Table S1). Together, these data provide evidence of synthetic lethality mediated by a cell-penetrating lupus anti-DNA antibody on cancer cells deficient in DNA repair and demonstrate the potential utility of 3E10 as a targeted cancer therapy for malignancies with DNA repair deficiencies. Importantly, the synthetic lethal effect of 3E10 on BRCA2-deficient cancer cells is in keeping with the mechanistic experiments described above, which show that 3E10 impacts BER and HDR.

We next hypothesized that 3E10 would have an even greater impact on BRCA2-deficient cancer cells when coupled with a DNA-damaging agent. BRCA2-deficient PEO1 and BRCA2-proficient PEO1 C4-2 ovarian cancer cells were treated with control buffer, 3E10, low dose doxorubicin, or 3E10 + low dose doxorubicin, and cell death was then evaluated by propidium iodide (PI) fluorescence three days later. The low dose of doxorubicin (3 nM) minimized cell killing by doxorubicin alone. As predicted, the combination of 3E10 and low dose doxorubicin was profoundly and selectively toxic to BRCA2-deficient cells (Figs. 4B–4D). Similarly, treatment of BRCA2-deficient CAPAN1 cells with 3E10 scFv combined with 2 Gy of IR reduced the surviving fraction from 0.48 ± 0.02 (SEM) with 2 Gy alone to 0.24 ± 0.04 (SEM) with the combination ($p=0.009$) (Table S1). These data demonstrate that combination therapy comprised of 3E10 and a DNA-damaging therapy has the potential to yield a high therapeutic index when used for the treatment of repair-deficient malignancies.

Discussion

We have discovered that a naturally occurring, cell-penetrating lupus anti-DNA antibody inhibits DNA repair, sensitizes cancer cells to DNA-damaging therapy *in vitro* and *in vivo*, and, as a single agent, is synthetically lethal to BRCA2-deficient human cancer cells.

Mechanistically, our work suggests that these phenomena stem from the ability of 3E10 to inhibit specific DNA repair pathways. There is substantial evidence that interference with BER is especially toxic to cells with BRCA2-deficiency (12, 18, 19), which is the basis for the investigative use of poly(ADP-ribose) polymerase (PARP) inhibitors in breast cancer (22). There is also emerging evidence that further suppression of HDR is synthetically lethal to BRCA2-deficient cells (20). Hence, the synthetic lethality of 3E10 in BRCA2-deficient human cancer cells can be attributed to its inhibition of BER and to its inhibition of key steps in HDR. Importantly, the inhibitory effect of 3E10 on BER and HDR is partial and not complete, which likely explains why 3E10 alone is not toxic to repair-proficient cells. Partial inhibition of BER and HDR by 3E10 is also likely responsible for the dramatic difference in the degree of sensitization to DNA-damaging therapy mediated by 3E10 in repair-deficient cells compared to repair-proficient cells, providing the basis for an advantageous therapeutic gain. Extensive dose-response and pharmacokinetic studies on 3E10 were beyond the scope of the present study, and the impact of 3E10 on BRCA2-deficient PEO1 xenografts could not be tested due to low PEO1 tumor take rates and a tendency of PEO1 tumors to spontaneously regress. Furthermore, we expect that 3E10 will also be effective against human cancer cells deficient in DNA repair due to defects in other genes, such as BRCA1 and the RAD51 paralogs, but this remains to be determined.

In addition to its implications for cancer therapy, our findings also open new avenues for exploration into SLE pathophysiology. SLE patients are at increased risk for malignancy (such as lymphoma and lung cancer) compared to control populations, but unexpectedly low incidence rates of breast, ovarian, and prostate cancers are observed in SLE. Specifically, the standardized incidence ratios, relative to control populations, for breast, ovarian, and prostate cancer in SLE are 0.76, 0.66, and 0.72, respectively (23, 24). The reason for the decreased incidence of these malignancies in SLE is unknown, and genome wide association studies have failed to identify any SLE specific markers that associate with reduced risk for breast cancer (25). Notably, breast, ovarian, and prostate cancer are frequently associated with mutations in BRCA2. Based on our present finding that a lupus autoantibody is synthetically lethal to BRCA2-deficient cancer cells, it is now tempting to speculate that circulating lupus autoantibodies in SLE patients provide protection against the development of BRCA2-mutated tumors. Furthermore, our findings raise the possibility that circulating lupus autoantibodies contribute to the intrinsic deficiencies in DNA repair and increased susceptibility to DNA-damaging agents characteristic of cells from SLE patients (26).

In summary, the application of a cell-penetrating anti-DNA antibody to the treatment of cancer is a completely new approach to antibody-based therapy, and we believe that 3E10 has potential for development as a targeted therapy for cancers deficient in DNA repair, either as a single agent or in combination with DNA-damaging therapy. Numerous familial and sporadic human malignancies such as breast, ovarian, pancreatic, and prostate cancers harbor deficiencies in DNA repair, and the promise that PARP-1 inhibitors have shown in clinical trials in the treatment of cancers with DNA repair deficiencies raises the possibility that 3E10 could also have a substantial clinical impact. 3E10 did not cause any adverse events in a previous FDA-approved Phase I clinical trial testing 3E10 as a possible vaccine for SLE (6), and a well-defined pathway for the development of monoclonal antibodies into human therapies has been established. It is therefore likely that 3E10 could rapidly transition from basic science studies to human clinical trials and provide the basis for a new cancer therapy. Moreover, our findings establish proof of principle for the application of a lupus anti-DNA antibody to cancer therapy and identify lupus autoantibodies as a potential source of new therapeutic agents.

Materials and Methods

Cell lines

Skov-3, MCF-7, U251, and U87 cell lines were obtained from the American Type Culture Collection (ATCC). CAPAN1/neo cells were provided by Dr. Sweasy. PEO1, PEO1 C4-2, and PEO4 cells were a gift from Dr. Taniguchi (21). U2OS cells stably transfected with the DR-GFP recombination substrate were a gift from Dr. Jasin. Cells were grown and maintained in Dulbecco's Modification of Eagles Medium (DMEM; Mediatech®) supplemented with 10% fetal bovine serum (FBS) (Skov-3, MCF-7, U251, U87, U2OS, PEO1, PEO1 C4-2, PEO4) or Roswell Park Memorial Institute-1640 (RPMI-1640) supplemented with 15% FBS and 2 mM L-glutamine (CAPAN1/neo) at 37° Celsius in 5% CO₂.

Production and purification of 3E10, 3E10 scFv, and Fv-Hsp70

3E10 was purified from hybridoma supernatant as previously described (2, 3). 3E10 scFv and Fv-Hsp70 were expressed in and secreted from *Pichia pastoris* and were purified from yeast supernatant as previously described (5, 7, 8). Protein concentrations were determined by Bradford assay.

Cell penetration assay

Skov-3 cells were treated with medium containing control buffer or 5 µM 3E10 scFv for 30 minutes. Medium was then withdrawn and cells were washed, fixed, and stained with an anti-Myc antibody as previously described (5).

In vitro radiosensitization assays

U251, MCF-7, or CAPAN1/neo cells were grown in 6- or 12-well plates. One day after plating, cells were treated with medium containing control buffer or 3E10 scFv (10 µM for U251, 0.25 µM for MCF-7 cells, and 50 µM for CAPAN1/neo cells), and then irradiated one hour later using the X-RAD 320 Biological Irradiator (Precision X-Ray) set at 320 kV, 12.5 mA, 2mm Al filter, 20×20 cm collimator, SSD 50.0 cm, with dose rate 2.43 Gy/min. Cells were then maintained in the same medium and incubated for colony formation. Surviving fractions were calculated relative to untreated controls assayed in the same experiment.

In vitro chemosensitization assays

U87 cells were grown in 96-well plates. One day after plating cells were treated with medium containing control buffer or 3E10 scFv (10 µM) and increasing doses of doxorubicin (0–250 nM) or paclitaxel (0–2.5 nM). Cells were then maintained in the same medium for one week, and the percentage of dead cells present was determined by propidium iodide (PI) exclusion assay, by measuring the percentage of cells with positive PI nuclear fluorescence (after confirming equivalent total cell numbers) as previously described (9).

In vivo radiosensitization experiment

Human glioma xenografts were implanted in 2–3 month old female athymic nude mice (Charles River Laboratories) by subcutaneous injection of U87 cells (6.0×10^6 cells in PBS). Mice were divided into 4 groups: “Control” (N=8), “Antibody” (N=8), “8 Gy” (N=8), and “8 Gy + Antibody” (N=7; one animal lost in anesthesia). After tumors had grown to a size of ~100 mm³ mice were treated with an intraperitoneal injection of control buffer (PBS; “Control” and “8 Gy” groups) or 3E10 (1 mg in PBS; “Antibody” and “8 Gy + Antibody” groups). Each group then received a second injection of the same reagent 24 hours later. 2 hours after the second injection, tumors in the “8 Gy” and “8 Gy + Antibody” groups were

irradiated with 8 Gy of localized tumor irradiation as previously described (27). Tumor volumes in each group were then followed and mice were sacrificed when tumor volume reached 1000 mm³. Tumor tripling time was calculated as the time required for tumors to increase in volume threefold over baseline (defined as tumor volume one day prior to treatment with antibody). P values were determined by Mann-Whitney rank sum test.

In vivo chemosensitization experiment

Human glioma xenografts were implanted in 4-week old C.B-17 SCID mice (Taconic) by subcutaneous injection of U87 cells (1.0X10⁷ cells in PBS). Mice were divided into 4 groups: “Control” (N=4), “Antibody” (N=4), “Dox” (N=4), and “Dox + Antibody” (N=4). After tumors had grown to a size of ~200 mm³ mice were treated with intraperitoneal injection of control buffer (PBS; “Control” group), 3E10 (0.8 mg in 0.5 mL PBS; “Antibody” group), PBS containing doxorubicin (80 µg/kg; “Dox” group), or doxorubicin (80 µg/kg) + 3E10 (0.8 mg in 0.5 mL PBS) (“Dox + 3E10” group). The IACUC protocol for this experiment required mice to be sacrificed when tumor volume reached 400 mm³, and tumors in the “Control”, “Antibody”, and “Dox” groups rapidly grew to size >400 mm³. The impact of each therapy was evaluated by comparing tumor growth over the three days after treatment. The P value was determined by paired two-tailed Student’s t-test.

DNA binding studies

³²P-radiolabeled DNA substrates (in conformations including single-stranded DNA, blunt-end duplex DNA, duplex DNA with an internal bubble due to heterology, duplex DNA with splayed single-stranded ends, duplex DNA with a 5’ single-stranded tail, and duplex DNA with a 3’ tail) were prepared as previously described (28). Each substrate was incubated with 3E10 (0–10 µM) for 30 minutes at 4° C, followed by electrophoretic mobility shift analysis as previously described (28). K_ds for substrates with and without a free single-strand tail were determined from aggregate plots of percent oligonucleotide bound (determined using ImageJ; National Institutes of Health) versus concentration of 3E10.

Single-strand break/base excision repair assay

The impact of 3E10 on BER was measured *in vitro* by examining the repair of a uracil in a synthetic radiolabeled duplex DNA substrate incubated with requisite repair enzymes including uracil DNA glycosylase, AP endonuclease, DNA polymerase β, and ligase in the presence of control buffer or 20 µM 3E10 or control anti-tubulin antibody. The repair reaction was stopped at the indicated time points, and the *n*, *n+1*, and *duplex* reaction products were quantified by gel electrophoresis and autoradiography as previously described (12).

Electron microscopy

M13 ssDNA (30 nM nucleotides) was incubated with 3E10 (30 nM) in 12.5 µl buffer EM (20 mM Tris-HCl, pH 7.5, 25 mM KCl, 1 mM DTT, 1 mM MgCl₂) for 30 min at 4°C. Purified RAD51 (0.5 µM) and 3E10 (2 or 4 µM) were incubated with 150-mer ssDNA (1.5 µM nucleotides) in 12.5 µl buffer EM containing 1 mM AMP-PNP for 10 min at 37°C. The specimens were adsorbed onto glow-discharged thin layer carbon-coated EM grids followed by staining with 2% (weight/volume) uranyl formate for 1 min and then air-dried. The grids were examined in an FEI Tecnai 12 electron microscope operated at 80 kV and images were recorded on a Gatan Ultrascan 4000 × 4000 CCD camera.

RAD51-mediated strand exchange assay

2 µM purified human RAD51 (hRAD51) or RAD51 variant hRAD51K133R (29) was incubated with an unlabeled 150-mer oligonucleotide (40 nM) in 12.5 µl Tris HCl (pH 7.5),

1 mM DTT, MgCl₂, 2 mM ATP, and 24 mM KCl for 5 min at 37°C. Then, 3E10 (0–35 µM) or control anti-His₆ (0–15 µM) antibody was added to the reaction and incubated for 5 min at 37°C. This was followed by the addition of ³²P-labeled homologous 40-bp dsDNA (40 nM) and spermidine (4 mM) and incubation for 30 min at 37°C. The strand exchange reaction was then stopped and deproteinized by the addition of SDS (0.5%) and proteinase K (80 ng/µl) and incubation for 10 min at 37°C. The degree of strand exchange was then visualized by electrophoresis in polyacrylamide gels at 4°C in TAE buffer (40 mM Tris acetate at pH 7.4, 0.5 mM EDTA) and quantified by autoradiography as previously described (30).

Immunofluorescence

Immunostaining for γH2AX was performed as previously described (12). The P value was determined by paired two-tailed Student's t-test.

Chromosome-based fluorescence assay of HDR efficiency

U2OS cells stably transfected with the DR-GFP recombination substrate were treated with control buffer or 10 µM 3E10 for 5 hours. Cells were then subjected to mock transfection or were transfected using the Amaxa Nucleofector with 4 µg of the I-SceI endonuclease and then maintained in control buffer or 10 µM 3E10. Forty-eight hours after this transfection cells were analyzed for GFP expression by fluorescence-activated cell sorting (FACS), as previously described (31). The P value was determined by paired two-tailed Student's t-test.

Synthetic lethality assays

Clonogenic assays: BRCA2-proficient (PEO1 C4-2) and BRCA2-deficient (PEO1 or CAPAN1/neo) cells were plated in 12-well plates. One day after plating, cells were treated with medium containing control buffer or 3E10 scFv (10 µM for PEO1 C4-2 and PEO1 cells, 50 µM for CAPAN1/neo cells). Cells were then incubated for colony formation, and surviving fractions were calculated relative to untreated control cultures from the same experiments. Luminescent cell viability assay: BRCA2-proficient (PEO4) and BRCA2-deficient (PEO1) cells were plated in 96-well plates. One day after plating cells were treated with 3E10 scFv (0–2 µM). Three days later cell viability was measured using the CellTiter-Glo® luminescent assay. PI exclusion assay: BRCA2-proficient (PEO1 C4-2) and BRCA2-deficient (PEO1) cells were plated in 96-well plates. One day after plating, cells were treated with medium containing control buffer or the full 3E10 antibody (0–10 µM). One week later, the percentage of dead cells in the population was determined by measuring the percentage of cells with positive PI nuclear fluorescence as described above. P values were determined by paired two-tailed Student's t-test.

Chemosensitization of BRCA2-deficient tumor cells

BRCA2-deficient PEO1 and BRCA2-proficient PEO1 C4-2 ovarian cancer cells were treated with control buffer, 10 µM 3E10, 3 nM doxorubicin (Dox), or 10 µM 3E10 + 3 nM doxorubicin. The percentage of dead cells in the population was then measured three days later using the PI exclusion assay as described above. A low dose of doxorubicin (3 nM) and short experiment time (three days) were selected to minimize the effect of doxorubicin or 3E10 alone on the cells. The P value was determined by paired two-tailed Student's t-test.

Statistics

Error bars represent standard error (SEM) or standard deviation (SD) of the mean of multiple experiments. P values were determined by paired two-tailed Student's t-test or Mann-Whitney rank sum test, where indicated.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. 3E10 sensitizes cancer cells to DNA-damaging therapy *in vitro* and *in vivo*
(A) 3E10 scFv penetrates into nuclei of Skov-3 ovarian cancer cells, as evidenced by anti-Myc immunostaining to detect the C-terminal Myc tag in 3E10 scFv. **(B)** 3E10 scFv sensitizes human breast cancer cells to ionizing radiation. Clonogenic survival curves for MCF-7 cells irradiated in the presence of control buffer, 3E10 scFv, or Fv-Hsp70 are presented. Error bars: SEM. N=5. **(C)** 3E10 scFv sensitizes human glioma cells to doxorubicin. U87 glioma cells were treated with doxorubicin (0–250 nM) in the presence or absence of 10 μ M 3E10 scFv, and the percentage of dead cells in the population was determined by propidium iodide fluorescence seven days later. Error bars: SD. N=9. **(D and E)** 3E10 sensitizes human glioma xenografts to IR *in vivo*. Treatment groups were: Control (N=8); Antibody (N=8), 8 Gy of IR (N=8), and 8 Gy + Antibody (N=7; one animal lost in anesthesia). Tumor growth measurements $\pm$ SEM are shown in panel 1D. Tumors were irradiated on day 27 (indicated by arrow). Kaplan-Meier plots of the percentage of tumors smaller than 3X baseline size are presented in panel 1E. Baseline size was defined as tumor size one day prior to antibody treatment (day 25 in panel 1D).

Fig. 2. 3E10 preferentially binds DNA substrates with a free single-strand tail and inhibits DNA single-strand break repair

(A) Schematic demonstrating the different DNA substrate conformations used for 3E10-DNA-binding assays. (B) DNA-binding curves comparing the binding affinity of 3E10 for substrates with and without a free single-strand tail. Error bars: SEM. N=12. (C and D) 3E10 inhibits single-strand break/base excision repair. The impact of 3E10 on BER was measured *in vitro* by examining the repair of a uracil in a synthetic radiolabeled duplex DNA substrate incubated with requisite repair factors in the presence of control buffer or 20 μM 3E10. The repair reaction was stopped at the indicated time points, and the n, n+1, and duplex reaction products were quantified by gel electrophoresis and autoradiography. Representative autoradiographs are shown in panel 2C and the proportion of the n+1 product over time in the presence or absence of 3E10 is presented in bar graph form in panel 2D. N=3.

Fig. 3. 3E10 inhibits homology-directed DNA double-strand break repair

(A) Scheme for *in vitro* strand exchange assay. See Materials and Methods for details. (B and C) 3E10 inhibits RAD51-mediated strand exchange. The impact of 3E10 (0–35 μ M) on RAD51-mediated strand exchange was tested using wild-type hRAD51 protein (panel 3B) or variant hRAD51K133R, which forms a stable presynaptic filament by attenuation of ATP hydrolysis and is an even more potent mediator of strand exchange (29) (panel 3C). Representative autoradiographs are shown, and the percentage strand invasion at increasing dose of 3E10 is presented in bar graph form. N=3.

(D and E) 3E10 scFv delays resolution of IR-induced DNA DSB in human glioma cells. U251 glioma cells were treated with control buffer or 3E10 scFv (10 μ M) and then irradiated with 2 Gy. Twenty-four hours later the numbers of DNA DSBs per cell were quantified by γ H2AX immunofluorescence. Representative immunofluorescence images are shown in panel 3D, and average number of γ H2AX foci per cell is quantified in panel 3E. Error bars: SEM. N=3.

Fig. 4. 3E10 is synthetically lethal to BRCA2-deficient cancer cells

(A) BRCA2-deficient PEO1 and BRCA2-proficient PEO1 C4-2 (a sub-clone of PEO1) human ovarian cancer cells were treated with the full 3E10 antibody (0–10 μM). The percentage of dead cells was then measured by assessing loss of membrane integrity via propidium iodide uptake and fluorescence 7 days later. Error bars: SEM. N=9. (B–D) 3E10 strongly and selectively sensitizes BRCA2-deficient cancer cells to low dose doxorubicin. BRCA2-deficient PEO1 and BRCA2-proficient PEO1 C4-2 ovarian cancer cells were treated with control buffer, 10 μM 3E10, 3 nM doxorubicin (Dox), or 10 μM 3E10 + 3 nM doxorubicin. The percentage of dead cells was then measured 3 days later via propidium iodide uptake and fluorescence. Propidium iodide fluorescence images are shown in panel 4B (uptake of fluorescent label indicates cell death), and percent cell death is quantified in panel 4C and 4D. Error bars: SEM. N=9.