Tumor and Stem Cell Biology

A Small Interfering RNA Screen of Genes Involved in DNA Repair Identifies Tumor-Specific Radiosensitization by POLQ Knockdown

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Abstract

The effectiveness of radiotherapy treatment could be significantly improved if tumor cells could be rendered more sensitive to ionizing radiation (IR) without altering the sensitivity of normal tissues. However, many of the key therapeutically exploitable mechanisms that determine intrinsic tumor radiosensitivity are largely unknown. We have conducted a small interfering RNA (siRNA) screen of 200 genes involved in DNA damage repair aimed at identifying genes whose knockdown increased tumor radiosensitivity. Parallel siRNA screens were conducted in irradiated and unirradiated tumor cells (SQ20B) and irradiated normal tissue cells (MRC5). Using γH2AX foci at 24 hours after IR, we identified several genes, such as BRCA2, Lig IV, and XRCC5, whose knockdown is known to cause increased cell radiosensitivity, thereby validating the primary screening end point. In addition, we identified POLQ (DNA polymerase θ) as a potential tumor-specific target. Subsequent investigations showed that POLQ knockdown resulted in radiosensitization of a panel of tumor cell lines from different primary sites while having little or no effect on normal tissue cell lines. These findings raise the possibility that POLQ inhibition might be used clinically to cause tumor-specific radiosensitization. Cancer Res; 70(7) April 1, 2010

Introduction

Radiotherapy is a vital tool in the management of cancer patients. It is often given with curative intent either alone or with chemotherapy in patients with diseases as diverse as head and neck, cervix, bladder, and non–small cell lung cancer. The radiation dose that can safely be delivered to patients is limited by the dose tolerances of surrounding normal tissues (1). It is anticipated that the effectiveness of radiotherapy would be improved if tumor cells could be rendered more sensitive to ionizing radiation (IR) without altering the sensitivity of normal tissues. Such a strategy depends on exploiting tumor-specific molecular targets, many of which remain to be identified. The intrinsic radiosensitivity of tumors differs significantly. Importantly, these variations in radiosensitivity have a large clinical effect, as those patients with radioresistant tumors are more likely to develop local recurrences (2–4) and have poorer survival rates (3, 4) than patients with more radiosensitive disease.

A recent trial in patients with locally advanced head and neck cancer compared combination treatment of cetuximab with radiotherapy against radiotherapy alone (5). This trial showed improved locoregional control and overall survival in patients treated with cetuximab and radiotherapy. Importantly, the addition of cetuximab was not associated with increased normal tissue toxicity. This important study has shown the potential improvements that can be achieved by specifically rendering tumor cells more sensitive to radiation therapy.

Previous attempts have been made to identify targets involved in radiosensitivity through the screening of small interfering RNA (siRNA) libraries. These studies have assessed radiation sensitivity using short-term assays based on cell viability (6, 7). This approach is potentially flawed as it may fail to distinguish between growth inhibition and clonal inactivation. The clonogenic survival assay is the “gold standard” method for assessing intrinsic radiosensitivity in vitro (8). Unfortunately, this assay is not suitable for use in large-scale siRNA screens due to the highly labor-intensive nature of the assay.

The critical role of DNA double-strand breaks (DSB) and chromosome aberrations produced by IR in causing cell death has long been recognized (9, 10). DSB formation results in rapid phosphorylation of histone H2AX (γH2AX). Typically, most of these γH2AX foci resolve within a few hours of irradiation. We reasoned that foci persisting at 24 hours may mark sites of delayed repair and could correspond to the sites likely to lead to chromosome breaks. Previous studies have shown...
the correlation between intrinsic radiosensitivity and the persistence of γH2AX foci 24 hours after radiation (11, 12).

In the present study, we describe a siRNA screen of 200 genes involved in DNA damage repair aimed at identifying genes whose knockdown causes increased tumor radiosensitivity. Using γH2AX foci at 24 hours after IR, we identified POLQ (DNA polymerase γ) as a potential tumor-specific target whose knockdown led to tumor cell–specific radiosensitization.

Materials and Methods

Cell culture. The tumor cells used were the SQ20B (laryngeal), T24 (bladder), PSN1 (pancreas), and HeLa (cervix) lines. The SQ20B cell line was obtained from Dr. Ralph Weichselbaum (University of Chicago, Chicago, IL) and has been described previously (13, 14). The other tumor cell lines were obtained from the American Type Culture Collection (ATCC). Two types of normal human fibroblast cells (MRC5 and POC) were used at early passage. MRC5 cells were obtained from the American Type Culture Collection (ATCC). Two types of normal human fibroblast cells (MRC5 and POC) were used at early passage. MRC5 cells were obtained from the ATCC. POC cells were established by us and have been described previously (13). Cells were cultured in DMEM containing 4.5 g/L glucose (Invitrogen) supplemented with 10% fetal bovine serum. All cultures were maintained at 37°C in water-saturated 5% CO2/95% air. Cells were regularly tested for the presence of Mycoplasma contamination (Mycoplasma, Lonza). Cell morphology was regularly checked to ensure the absence of Mycoplasma contamination (MycoplasmaAlert, Lonza). Cell morphology was regularly checked to ensure the absence of cross-contamination of cell lines.

RNAi library and siRNA. A custom-designed DNA repair gene library of 200 pools of four siRNA strands (Supplementary Table S1) was used for the screen (siGenome, Dharmacon). In addition to the library wells, each plate contained four replica wells with nontargeting (NT) siRNA and four wells with DNA-PKcs siRNA as negative and positive controls, respectively. Individual genes were investigated using both pools and individual siRNAs (ON-TARGETplus, Dharmacon). An initial screen was separately conducted with both tumor cells (SQ20B) and normal tissue cells (MRC5) to identify genes whose knockdown caused tumor-specific radiosensitization. SQ20B and MRC5 cells were both reverse transfected with siRNA (final concentration, 50 nmol/L) using DharmaFECT 1 as per the manufacturer’s instructions in four replica 96-well plates. Forty-eight hours after transfection, the medium was replaced with DMEM with 10% fetal bovine serum. For both cell types, two replica plates were treated with 4 Gy using an IBL634 cesium irradiator at a dose rate of 0.66 Gy/min, and two plates were left unirradiated. Optimization studies showed that this dose of radiation resulted in sufficiently large differences in γH2AX foci formation between positive and negative controls. The SQ20B and MRC5 cells were irradiated 48 and 66 h, respectively, after transfection. After irradiation, the cells were returned to the incubator for 24 h. Cells were then fixed using 3% paraformaldehyde diluted in PBS before analysis of γH2AX foci.

Analysis of γH2AX foci. The techniques used to quantify γH2AX foci have been described previously (15). Briefly, after fixation, the cells were permeabilized and blocked with 0.1% Triton (v/v) diluted in PBS containing 1% bovine serum albumin (Sigma) for 1 h at room temperature. Cells were incubated with a primary mouse monoclonal antibody to γH2AX (1:1500; Millipore) overnight at 4°C. Cells were then washed thrice with PBS before incubation with Alexa Fluor 488–conjugated secondary antibody (1:1200; Invitrogen) for 1 h at room temperature. Cells were again washed thrice with PBS for 5 min before 4′,6-diamidino-2-phenylindole (DAPI) staining, 0.5 μg/mL diluted with PBS, for 10 min. The DAPI was replaced with PBS before foci were detected using an IN Cell Analyzer 1000 automated epifluorescence microscope (GE Healthcare). Four images were obtained per well. Foci quantitation was accomplished using IN Cell Analyzer Workstation software (v3.5). For unirradiated cells, the readout was the mean number of γH2AX foci per cell. For irradiated cells, this was the proportion of cells that contained more than seven γH2AX foci per cell. This was because optimization studies showed that analysis based on this technique correlated best with results obtained from clonogenic survival assays.

Clonogenic assay. For the clonogenic assays, all cell types were forward transfected in six-well plates with 50 nmol/L siRNA using DharmaFECT 1 (Dharmacon). In all clonogenic survival experiments, cells were plated 48 h after transfection from single-cell suspensions and allowed to adhere to culture dishes before irradiation with an IBL634 cesium irradiator at a dose rate of 0.66 Gy/min. Remaining cells from the transfection were used for quantitative reverse transcription-PCR (qRT-PCR) to confirm effective knockdown. Colonies were stained with crystal violet and counted 9 to 16 d after irradiation. Colony counting was primarily accomplished using an Oxford Optronics Colcount. Some primary cells formed diffuse colonies and required manual scoring. Each point on the survival curve represents the mean surviving fraction from four dishes. Clonogenic survival curves are representative of independent replicate experiments.

The surviving fraction was derived using the following formula:

\[ \text{Surviving Fraction} = \frac{\text{# of colonies}}{\text{# of cells plated}} \]

Experimental data were fitted with the linear quadratic model:

\[ S = \exp(-\alpha D - \beta D^2) \]

where \( S \) is the survival probability, \( D \) is the radiation dose (Gy), and \( \alpha \) and \( \beta \) are the fit parameters (Gy\(^{-1}\) and Gy\(^{-2}\), respectively).

The sensitization enhancement ratio (SER) was used to quantify radiosensitization (the SER10 was deduced from data by using SER10 = \( D_{\text{control}} / D_{\text{treated}} \), where \( D_{\text{control}} \) and \( D_{\text{treated}} \) doses yield 10% survival for controls and treated cells, respectively).

Drug treatment. For clonogenic assays, cells transfected with either POLQ or NT siRNA were allowed to adhere before addition of temozolomide (Sigma) at the stated concentrations for 2 h. The cells were then washed with PBS before the addition of complete medium and incubated for 14 d until colony staining. For γH2AX foci quantification, cells were plated in 96-well
Figure 1. Screening of a siRNA library of genes involved in DNA repair in SQ20B and MRC5 cells. A, irradiated SQ20B cells. Z-scores of the top 30 genes associated with elevated γH2AX foci 24 h after 4-Gy radiation. B, irradiated MRC5 cells. Z-scores of the top 30 genes associated with elevated γH2AX foci 24 h after 4-Gy radiation. C, radiosensitization of MRC5 cells with APEX2 depletion. Clonogenic assay in MRC5 cells treated with 50 nmol/L NT or APEX2 siRNA. **, P < 0.01, unpaired two-sided t test (left). Demonstration of effective knockdown of APEX2 by qRT-PCR. Right, gene expression normalized to cells treated with NT siRNA. D, unirradiated SQ20B cells. Z-scores of the top 30 genes associated with elevated γH2AX foci in cells transfected with siRNA pools.
plates as described above. In the experiments indicated, 48 h after forward transfection, the cells were treated with temozolomide for 2 h, at which point they were either left unirradiated or treated with 4 Gy. One hour after IR, the cells were washed thrice with PBS. Complete medium was then added, and the cells returned to the incubator until fixation 24 h after IR.

Quantification of gene silencing. RNA was extracted and purified from cells at the times indicated using the RNeasy Mini kit (Qiagen) as per the manufacturer’s instructions. One step qRT-PCR was performed on 500 ng RNA using SuperScript III Platinum SYBR Green One-step qRT-PCR kit (Invitrogen). The primers used for each gene are as follows: POLQ, TATCTGCTGGAACTTTTGCTGA (forward) and CTCA-CACCATTCTTTGTATGGA (reverse); APEX2, CTGTAAGGACAATGCTACCC (forward) and ACACGTTGATTAGGGTCAAG (reverse); and GAPDH, CCACCCATGGCAAATTCCATGGCA (forward) and TCTAGACGGCAGGTCAGGTCCACC (reverse).

qRT-PCR was achieved using a Stratagene Mx3005P system. cDNA synthesis was performed by heating the reagents to 42°C for 30 min followed by 95°C for 10 min. The amplification was performed at the following conditions for all three genes of interest: 95°C for 30 s, 58°C for 30 s, and 72°C for 60 s for 40 cycles.

Statistical analysis. Data were analyzed using the CellHTS2 package (16) as follows. Sample values from each plate were first normalized using the median of the NT siRNA control wells for each plate. Z-scores for each gene in each replicate were then calculated using the following formula: \( Z\text{-score} = \frac{\text{Sample}_{\text{norm}} - \text{Median}_{\text{NT}}}{\text{MAD}_{\text{NT}}} \), where \( \text{Sample}_{\text{norm}} \) is the normalized sample value and \( \text{Median}_{\text{NT}} \) and \( \text{MAD}_{\text{NT}} \) are the median and the median absolute deviation (MAD) of all NT control wells across all three library plates, respectively. The final Z-score was then calculated using the mean of the replicate Z-scores for each gene.

For clonogenic assays, unpaired \( t \) tests were conducted at each radiation dose exposure to assess differences in surviving fractions. All tests of significance were two-sided, and values of \( P < 0.05 \) were considered significant.

Results

A siRNA screen identifies genes potentially involved in tumor cell radiosensitivity. We screened a custom siRNA library of 200 genes involved in DNA repair using the radiosensitive SQ20B cell line as well as the MRC5 fibroblast line to identify novel tumor-specific radiosensitizing targets. The screen of irradiated SQ20B cells was used to compile a list of genes that may be involved in tumor-specific radiosensitivity. The magnitude of the Z-scores obtained in each of the screens differed significantly. In view of this, we decided the most practical way to define genes of interest was to examine the top 30 genes with the highest Z-scores. The Z-scores of the top 30 genes associated with elevated \( \gamma H2AX \) foci in SQ20B cells 24 hours after 4-Gy radiation are shown in Fig. 1A. Several of the genes identified in this screen are already known to increase cell radiosensitivity following knockdown. These included genes involved in homologous recombination, such as \( BRCA1 \) (17), \( BRCA2 \) (18), and \( RBBP8 \) (19), as well as genes involved in nonhomologous end joining, such as \( Lig IV \) (20), \( XRCC5 \) (Ku80; ref. 21), and \( PRKDC \) (DNA-PKcs; ref. 22). Genes involved in DNA damage response, such as \( 53BP1 \) (23), which is involved in cell radiosensitivity, were also identified by the screen. Depletion of TIMELESS, which was also associated with a high Z-score, has previously been shown to increase radiosensitivity but through mechanisms that are less clear (24). Of the remaining genes that have not previously been shown to be involved in tumor radiosensitivity, we decided
to investigate POLQ, RAD21, APEX2, and XAB2 in more detail, as these genes were considered to have clinically exploitable potential if it was shown that depletion of these genes caused tumor-specific radiosensitization.

The screen of irradiated MRC5 cells was used to filter the list of candidate genes. To identify candidate genes whose depletion sensitized tumor cells to radiation without affecting normal tissue radiosensitivity, we screened a fibroblast cell line with the same pools of siRNAs that were used to transfect the tumor cells. Figure 1B shows the top 30 genes associated with elevated γH2AX foci in irradiated MRC5 cells. One of the genes identified by this normal tissue screen was APEX2, which also had a high Z-score on the screen of irradiated tumor cells. As APEX2 has not previously been shown to be involved in intrinsic radiosensitivity, we performed clonogenic assays with MRC5 cells depleted of APEX2 and found that effective knockdown did indeed cause increased cell radiosensitivity (Fig. 1C). In view of these findings on a normal tissue line, this gene was not investigated further as a potential tumor-specific radiosensitizer. Supplementary Table S2 lists the genes that featured in the top 30 Z-scores of both the irradiated SQ20B and MRC5 cell line screens.

Identification of genes important in tumor cell survival independently of radiation. Of the remaining candidate genes being investigated, we tried to use the screen of unirradiated SQ20B cells as a filter to exclude those genes whose knockdown affected cell viability in the absence of exposure to IR. The Z-scores of the top 30 genes associated with elevated γH2AX foci in unirradiated SQ20B cells are shown in Fig. 1D. The effect of gene knockdown on cell survival has not previously been studied for several of these genes. Colony-forming assays performed with unirradiated SQ20B cells transfected with two of the siRNA pools ranked highly in this unirradiated screen (RPA1 and INCENP) siRNA pools resulted in widespread cell death and no colony formation (data not shown). These initial results suggested that the screen could be used as an effective filter for siRNAs, which caused cell death in unirradiated conditions. Supplementary Table S3 lists those genes that featured in the top 30 Z-scores of the irradiated SQ20B but not in either the irradiated MRC5 screen or the unirradiated SQ20B screen.

None of the remaining genes being investigated as causing tumor-specific radiosensitization (POLQ, RAD21, and XAB2) was associated with high Z-scores in the screen of unirradiated tumor cells. However, colony-forming assays performed with a panel of unirradiated cells transfected with both RAD21 and XAB2 siRNAs showed that knockdown of these genes resulted in widespread cell death (Supplementary Table S4). Therefore, the absence of elevated γH2AX foci in unirradiated cells transfected with pools of siRNA cannot reliably be used to predict the survival of cells in the absence of IR. However, the exclusion of those genes that cause γH2AX foci in the absence of radiation may reduce the number of false-positive genes in the screen of irradiated cells. Having excluded three of the four genes initially identified by the screen of irradiated SQ20B cells, we investigated the remaining gene, POLQ, in more detail.

**POLQ knockdown is associated with increased γH2AX foci following IR.** POLQ (DNA polymerase θ) is a low-fidelity
DNA polymerase with limited normal tissue expression, whose normal physiologic functions are largely unknown. POLQ featured in the top 30 Z-scores of the irradiated SQ20B screen but not in the screens using irradiated MRC5 cells or unirradiated SQ20B cells, suggesting that it does not affect MRC5 cell survival after irradiation or SQ20B cell viability in the absence of irradiation. As POLQ knockdown has not previously been shown to sensitize human tumor cells to IR, this gene was investigated further. First, we aimed to replicate the results found in the screen. SQ20B cells were transfected in triplicate wells of two 96-well plates with either NT or POLQ siRNA. One plate was irradiated with 4 Gy and the other was left unirradiated. Twenty-four hours after IR, cells were analyzed for γH2AX foci. Unirradiated cells with POLQ knockdown did not have increased γH2AX foci compared with negative controls (Fig. 2A). However, the irradiated cells treated with POLQ siRNA had significantly increased residual γH2AX foci compared with irradiated cells treated with NT siRNA (Fig. 2B and C).

**POLQ knockdown sensitizes several tumor cell lines to IR.** To confirm that the observed increase in γH2AX foci associated with irradiated SQ20B cells depleted of POLQ translated to an increase in tumor cell radiosensitivity, we performed clonogenic assays with the SQ20B cells used in the primary screen along with a second tumor line (HeLa) following transfection with either NT or POLQ siRNA. Figure 3 confirms that both cell lines were sensitized to IR by POLQ knockdown. Clonogenic assays performed on a third tumor (T24 transitional cell bladder carcinoma) confirmed that the effects of POLQ knockdown were not restricted to squamous cell carcinomas.

To confirm that the observed results did not occur as a result of off-target effects, we repeated the experiment with individual siRNAs as well as the siRNA pool (Fig. 4A). The individual siRNA strand transfections were performed with a final concentration of 25 nmol/L. The degree of POLQ knockdown was well correlated with the magnitude of associated radiosensitization, strongly suggesting that the observed effects with POLQ siRNA did not occur as a result
of off-target effects. Of the four individual siRNAs, strand “1” caused both the most potent silencing and radiosensitization (Fig. 4A and B).

**POLQ knockdown causes minimal effects on normal tissue radiosensitivity.** Previous work has shown that POLQ expression is limited to only a few normal tissues (25). To confirm that treatment with POLQ siRNA did not significantly alter the radiosensitivity of normal tissue cell lines, we performed clonogenic assays on two fibroblast lines: MRC5 and POC cells. The POC cells did not express POLQ (data not shown), and treatment with POLQ siRNA had no radiosensitizing effects (Fig. 5A). As well as supporting the hypothesis that POLQ knockdown may cause tumor-specific radiosensitization, this also confirms that the observed effects did not occur as a result of off-target effects. In the MRC5 cells, POLQ siRNA treatment caused only a marginal increase in MRC5 radiosensitivity at very high doses of radiation (Fig. 5B and C). A comparison between POLQ expression normalized to the expression of a housekeeping gene (GAPDH) in untransfected cells showed that MRC5 cells express POLQ at a level ~50 times lower than the T24 tumor cell line (Fig. 5D). In unirradiated cells, POLQ knockdown did not consistently reduce colony formation in either the normal tissue or the tumor cell lines (Supplementary Table S5).

**POLQ knockdown has no effects on cell response to temozolomide with or without IR.** Temozolomide is an orally available alkylating agent that has an established role in the treatment of glioblastomas (26). It has previously been shown that a significant proportion of the DNA damage caused by temozolomide is repaired by the base excision repair (BER) pathway and that cells with deficiencies in the BER pathway have increased sensitivity to temozolomide (27). As it has previously been suggested that POLQ plays a role in BER, we examined whether POLQ knockdown rendered cells more sensitive to temozolomide. However, clonogenic assays performed on SQ20B cells treated with either POLQ or NT siRNA showed no difference in sensitivity to drug treatment with temozolomide (Fig. 6A and B).

For both unirradiated (Fig. 6C) and irradiated conditions (Fig. 6D), SQ20B cells treated with temozolomide were found to have elevated γH2AX foci 24 hours after drug exposure compared with cells not exposed to the drug. However, this occurred with the same magnitude regardless of whether cells had been transfected with either NT or POLQ siRNA. These findings would suggest that the mechanism by which POLQ knockdown causes increased radiosensitivity are independent of BER.

**Discussion**

The known DNA repair defects that produce large differences in radiation sensitivity are often associated with complex clinical syndromes, such as ataxia-telangiectasia, Nijmegen breakage syndrome, and Fanconi anemia (28). The proteins identified by these repair defects do not represent potential therapeutic targets due to lack of tumor specificity. Many of
the key therapeutically exploitable mechanisms that determine intrinsic tumor radiosensitivity are largely unknown. The clinical importance of these mechanisms is shown by the known correlation between increased tumor radioresistance and adverse patient outcomes (2–4).

The epidermal growth factor receptor pathway is the most widely studied contributor to tumor cell radioresistance. Recent trials have shown the large benefits that can potentially be derived from biological treatments that selectively render tumor cells more sensitive to radiation by manipulation of this pathway and illustrate the need for greater understanding of the molecular basis for tumor radioresistance (5, 29).

This siRNA screen of genes involved in DNA repair was based on the critical role that unrepaired DSBs play in cell death following IR. Of the genes whose knockdown was associated with increased γH2AX foci in SQ20B cells following IR, several have already been shown to be associated with increased cell radiosensitivity (17–23), thus validating the primary screening end point. The experimental design used both irradiated and unirradiated tumor cells as well as parallel siRNA screens in a tumor line and normal tissue line, allowing the identification of siRNAs that cause tumor-specific radiosensitization. High-throughput screens will inevitably feature both false-positive and false-negative results. Failure to cause sufficient gene knockdown is one of the most common causes for obtaining false-negative results. *ATM* was one of the genes included in this library, which is involved in cell radiosensitivity but which was not in the top 30 Z-scores of the irradiated cell screens. In this case, it is probable that this occurred because *ATM* is one of the genes involved in causing H2AX phosphorylation in response to DSB formation (30) and, thus, is a false negative in this assay.

Of the targets identified by this screen, we elected to investigate *POLQ* further as this gene has not previously been linked to tumor cell radiosensitivity and because a previous study has suggested a potentially exploitable difference in expression between normal tissues and tumor cells (25).

*POLQ* is a member of the A family of DNA polymerases, which, unusually for this class of polymerases, synthesizes DNA with very low fidelity (31, 32). The normal physiologic functions associated with this protein are currently unclear. It has previously been suggested that *POLQ* plays a dominant role in the process of somatic hypermutation of immunoglobulin genes. This suggestion arose from the observation that mice deficient in *POLQ* had a substantially decreased frequency of mutations in immunoglobulin genes (33). A separate group also found a decrease in mutation frequency in *POLQ*−/− mice but to a lesser degree (34). However, a recent study found that mutation types and frequencies were similar in wild-type, *POLQ*−/−, *POLH*−/−, and *POLQ*−/−*POLH*−/− mice (35). Accordingly, this group suggested that *POLQ* does not have a significant role in the hypermutation pathway.

It has been suggested that *POLQ* has a role in BER, but this remains unresolved. Mutation of *POLQ* in the DT40 chicken B-cell lymphocyte line has been shown to increase sensitivity to H$_2$O$_2$. *POLQ/POLβ* mutants had significantly

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**Figure 6.** Effect of temozolomide treatment after *POLQ* knockdown. 
A, clonogenic assays performed with SQ20B cells transfected with either NT or *POLQ* siRNA. Survival following temozolomide treatment expressed as a fraction relative to cells not exposed to temozolomide. B, confirmation of effective knockdown of *POLQ* in SQ20B cells as determined by qRT-PCR. C, effects of temozolomide treatment on the percentage of unirradiated SQ20B cells containing more than seven γH2AX foci per cell. D, percentage of SQ20B cells containing more than seven γH2AX foci per cell following 4-Gy irradiation.
higher sensitivity to methyl methanesulfonate than either single mutant. Extracts obtained from this cell line were used to show that POLQ mutant cells have markedly reduced single-nucleotide BER capacity in vitro and that this reduction was of a similar magnitude to cells deficient in POLβ (36). These findings led to the suggestion that POLQ and POLβ cooperate in BER.

Recent biochemical analysis has looked at the in vitro activity of cloned human POLQ (37). It was shown that full-length POLQ has 5′-deoxyribose phosphate (5′-dRP) lyase activity. A COOH-terminal fragment of POLQ was shown to carry 5′-dRP lyase activity, and this seemed to be independent of polymerase activity. The full-length protein and the COOH-terminal fragment were shown to have BER activity in vitro. Although these findings have been used to support the argument that POLQ may have a role in BER in vivo, it should be noted that the rate of 5′-dRP lyase activity of POLQ is ~40-fold slower than that of POLβ. We found that POLQ knockdown did not alter the sensitivity of cells to temozolomide either with or without IR. We interpret this to mean that the mechanism by which POLQ knockdown causes increased sensitivity to IR is independent of BER, although it remains possible that POLQ facilitates repair via BER of a lesion that is produced by IR but not by temozolomide.

POLQ expression was previously assessed by RT-PCR in a variety of different normal human tissues (25). Interestingly, expression was primarily limited to lymphoid tissues such as the fetal liver, thymus, and bone marrow. Critical normal tissues such as lung, liver, small intestine, kidney, heart, brain, and spinal cord that typically limit the radiation dose that can be delivered to patients did not seem to express POLQ. This would imply that inhibition of POLQ would not alter the intrinsic sensitivity of these tissues. Intriguingly, this study also found that POLQ was overexpressed in a large proportion of tumors derived from patients with colon, lung, and gastric cancer. Given the findings presented here, POLQ inhibition in these tumors would be predicted to reduce their radiation survival.

This difference in expression of POLQ in tumor cells and critical, radiosensitive normal tissues was central to our decision to investigate POLQ further. Although it is possible that depletion of POLQ from the small number of normal tissues that express this protein may render these cells more sensitive to radiation (38), the very restricted normal tissue expression means that POLQ inhibition may improve the therapeutic ratio of radiotherapy.

Our findings confirm that POLQ is overexpressed in tumor cells derived from a variety of primary sites and that POLQ knockdown causes increased intrinsic radiosensitivity. Our results are consistent with the limited normal tissue expression of POLQ and, therefore, that depletion of POLQ might cause much less radiosensitization of normal tissues compared with tumors. These findings raise the possibility that POLQ inhibition could be used clinically to cause tumor-specific radiosensitization. Additionally, the technique used in this study successfully identified several other genes already known to play a role in intrinsic radiosensitivity, thus validating its use in future screens of larger siRNA libraries.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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