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Brief report

A high-throughput RNA interference screen for DNA repair determinants of PARP inhibitor sensitivity

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ABSTRACT

Synthetic lethality is an attractive strategy for the design of novel therapies for cancer. Using this approach we have previously demonstrated that inhibition of the DNA repair protein, PARP1, is synthetically lethal with deficiency of either of the breast cancer susceptibility proteins, BRCA1 and BRCA2. This observation is most likely explained by the inability of BRCA deficient cells to repair DNA damage by homologous recombination (HR) and has led to the clinical trials of potent PARP inhibitors for the treatment of *BRCA* mutation-associated cancer. To identify further determinants of PARP inhibitor response, we took a high-throughput genetic approach. We tested each of the genes recognised as having a role in DNA repair using short-interfering RNA (siRNA) and assessed the sensitivity of siRNA transfected cells to a potent PARP inhibitor, KU0058948. The validity of this approach was confirmed by the identification of known genetic determinants of PARP inhibitor sensitivity, including genes involved in HR. Novel determinants of PARP inhibitor response were also identified, including the transcription coupled DNA repair (TCR) proteins DDB1 and XAB2. These results suggest that DNA repair pathways other than HR may determine sensitivity to PARP inhibitors and highlight the likelihood that ostensibly distinct DNA repair pathways cooperate to maintain genomic stability and cellular viability. Furthermore, the identification of these novel determinants may eventually guide the optimal use of PARP inhibitors in the clinic.

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1. Introduction

Cellular DNA is constantly exposed to multiple insults that have the potential to disrupt genomic integrity, affect cell viability and cause tumourigenesis. The deleterious effects of DNA damage are, in part, limited by a series of molecular mechanisms that have evolved to repair this damage. Mammalian cells have four main DNA repair pathways, which, ostensibly deal with distinct forms of DNA damage [1]. DNA damage that affects only a single DNA strand and which does not significantly disrupt the helical structure of DNA, such as oxidative damage, deamination and single strand DNA breaks (SSBs), is generally repaired by the base excision repair (BER) pathway. DNA lesions that damage a single DNA strand but distort the DNA helix, such as those caused by UV light and agents such as polycyclic aromatic hydrocarbons,

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are repaired by nucleotide excision repair (NER). Double strand DNA breaks (DSBs), caused by, for example, exposure to ionising radiation, are repaired by homologous recombination (HR), non-homologous end-joining (NHEJ), or single strand annealing (SSA). Finally, mismatches in the base pairing of DNA, caused by replication errors, are repaired by the mismatch repair (MMR) pathway [1].

While this simplistic model suggests that particular types of DNA damage are processed by distinct pathways, there is, in fact considerable interaction and overlap between DNA repair pathways. Oxidative damage of DNA, for example, can be repaired by both BER and NER [2]. Furthermore, failure of one DNA repair pathway is often compensated by the action of another, such as when BER fails to repair a SSB, HR is able to compensate [3]. Our previous work [3,4] and that of others [5] has exploited the interplay between DNA repair pathways to design a novel therapeutic approach to the treatment of breast cancer. Cells with deficiencies in the breast cancer susceptibility proteins BRCA1 or BRCA2 are profoundly sensitive to potent inhibitors of the DNA repair enzyme poly (ADP)-ribose polymerase 1 (PARP) [3]. We have subsequently demonstrated that this effect is most likely due to a deficiency in DNA repair by the process of HR, as deficiency in other proteins required for fully functional HR (RAD51, RAD54, DSS1, RPA1, NBS1, ATR, ATM, CHK1, CHK2, FANCD2, FANCA, and FANCC) also sensitizes to the drug-like PARP inhibitor KU0058948 [6]. The degree of selectivity that KU0058948 has for cells with defective HR is of such a magnitude – BRCA2 deficient cells are up to 1000-fold more sensitive to KU0058948, compared to isogenically-matched wild type cells [3] – that targeting tumours with deficiencies in HR by the use of PARP inhibitors is a promising therapeutic approach [7-9]. Clinical trials to test the efficacy of this strategy for the treatment of cancer are currently underway [10].

The profound sensitivity of HR deficient cells to PARP inhibitors is likely to be due to the role that PARP plays in SSB repair. Inhibition of PARP results in a persistence of SSBs [11]. Persistent SSBs, when encountered by replication forks in the S phase of the cell cycle, may lead to the collapse of the replication fork and the formation of potentially lethal DSBs [3]. As HR is primarily concerned with the error-free repair of DSBs at replication forks [12], we hypothesised that cells with HR deficiency are particularly sensitive to PARP inhibitors as they are unable to effectively cope with this increase in lethal DSBs associated with replication fork collapse [3]. The observation that one DNA repair mechanism (HR) compensates for deficiencies in another (SSB repair) may suggest that other novel therapeutic approaches could be developed that exploit the simultaneous inhibition of different pathways of DNA repair. Furthermore, the functional interplay between SSB repair and HR suggests that there is significant interaction between what are superficially distinct mechanisms of DNA repair and is an example of a synthetic lethal relationship [13].

Given the promise of PARP inhibition as a therapeutic approach, it is important to detail the molecular determinants of response to PARP inhibitors. Identifying novel determinants of response to a candidate clinical agent might extend their utility, as well as providing potential insight into drug resistance mechanisms. Recent studies have demonstrated that novel kinase determinants of PARP inhibitor sensitivity, such as *CDK5*, can be identified using high-throughput RNA interference screening [14]. Here we describe a similar genetic approach to the identification of DNA repair genes that determine PARP inhibitor sensitivity.

2. Materials and methods

2.1. Cell lines and compounds

CAL51 cells were obtained from ATCC (USA) and maintained in DMEM (Sigma, Poole, UK) supplemented with 10% FCS (10%, v/v) glutamine and antibiotics. The PARP inhibitor (KU0058948 – IC_{50} 3.2 nM) has been described previously [3].

2.2. RNAi library and siRNA

We used the Human DNA Repair siRNA Set V1.0 siRNA library (Qiagen, UK) arrayed in 6× 96-well plates. As a positive control for the HTS, siBRCA1 (D-003461, Dharmacon, USA) was used and siCONTROL*1 (siCON, D-001210-01, Dharmacon, USA) was used as a negative control. siRNA targeting BRCA2 (D-003462, Dharmacon, USA) was also used. Each plate contained 10 replica wells of siCON, four replica siBRCA1 wells and two blank wells, for use as “untransfected” controls.

2.3. HTS screen method

CAL51 cells plated in 96 well plates were transfected 24 h later with siRNA (final concentration 100 nM), using Lipofectamine 2000 (Invitrogen, USA) as per manufacturer’s instructions. Twenty-four hours following transfection cells were trypsinised and divided into six identical replica plates (1000 cells per well). At 48 h following transfection, three replica plates were treated with 0.01% (v/v) DMSO vehicle in media and three replica plates with 1 mM KU0058948 (PARP inhibitor) in media. Media containing KU0058948 or vehicle was replenished after 48 h, and cell viability was assessed after five days KU0058948 exposure using CellTiter-Glo® Luminescent Cell Viability Assay (Promega, USA) as per manufacturer’s instructions. The screen was completed in triplicate, after rejecting plates from the screen if mean growth in siCONTROL (siCON) wells was less than 60% of un-transfected control wells.

For each transfection the following were calculated:

Cell growth. The effect of each individual siRNA upon cell growth alone was calculated from DMSO treated cells by dividing mean luminescence in the three replica wells transfected with siRNA by mean luminescence of the replica wells transfected with siCON, and expressed as a percentage:

$$\text{cell growth effect of siRNA(\%)} = \frac{\text{mean luminescence (3 replica wells with siRNA)}}{\text{mean luminescence (12 replica well with siCON)}} \times 100$$

PARP inhibitor sensitivity. Sensitivity to PARP inhibitor for each siRNA was assessed by calculating the surviving fraction

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