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Chemical inhibitor targeting the replication protein A–DNA interaction increases the efficacy of Pt-based chemotherapy in lung and ovarian cancer

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ABSTRACT

Platinum-based chemotherapeutics exert their therapeutic efficacy via the formation of DNA adducts which interfere with DNA replication, transcription and cell division and ultimately induce cell death. Repair and tolerance of these Pt-DNA lesions by nucleotide excision repair (NER) and homologous recombination (HR) can substantially reduce the effectiveness of therapy. Inhibition of these repair pathways, therefore, holds the potential to sensitize cancer cells to Pt treatment and increase clinical efficacy. Replication Protein A (RPA) plays essential roles in both NER and HR, along with its role in DNA replication and DNA damage checkpoint activation. Each of these functions is, in part, mediated by RPA binding to single-stranded DNA (ssDNA). Here we report the synthesis and characterization of novel derivatives of RPA small molecule inhibitors and their activity in models of epithelial ovarian cancer (EOC) and non-small cell lung cancer (NSCLC). We have synthesized analogs of our previously reported RPA inhibitor TDRL-505 and determined the structure–activity relationships. These data led us to the identification of TDRL-551, which exhibited a greater than 2-fold increase in *in vitro* activity. TDRL-551 showed synergy with Pt in tissue culture models of EOC and *in vivo* efficacy, as a single agent and in combination with platinum, in a NSCLC xenograft model. These data demonstrate the utility of RPA inhibition in EOC and NSCLC and the potential in developing novel anticancer therapeutics that target RPA–DNA interactions.

1. Introduction

Platinum (Pt)-based combination chemotherapy has been the front-line treatment for a variety of malignancies including testicular, lung, and ovarian cancer [1]. However, resistance to Pt-based regimens remains a major limitation in the successful treatment for many of these cancers including epithelial ovarian cancer (EOC) and non-small cell lung cancer (NSCLC) [2,3]. More than 80% of EOC patients relapse with Pt-resistant disease, where second line therapies are largely ineffective. Thus, ovarian cancer has been clinically designated as the most deadly gynecological cancer owing to extremely poor prognosis and overall low survival rates [4]. The clinical efficacy of cisplatin is a function of its ability to cross-link DNA thereby blocking DNA replication, transcription and cell division. Ultimately Pt-treatment induces apoptosis [5,6], however, the balance between DNA damage and DNA repair dictates the extent of tumor death. While Pt-resistance is multifactorial, increased DNA repair is a major contributor [7]. Hence, exploiting DNA repair as a target to sensitize cells to Pt-based chemotherapy holds immense potential for increasing the survival rates in cancer therapy.

Repair and tolerance of cisplatin-DNA adducts occur primarily via nucleotide excision repair (NER) and homologous recombination (HR) [4,8,9]. Approximately 95% of Pt-DNA lesions formed by cisplatin are intrastrand crosslinks with the remaining ~5% being interstrand crosslinks and a small number of mono-lesions [10]. There is evidence for and against each lesion type being the cytotoxic lesion caused by cisplatin. Interstrand lesions are less abundant and repaired more efficiently than intrastrand lesions [11,12], and involve the HR pathway in conjunction with the FANC protein complex (a group of proteins associated with Fanconi

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anemia) [13]. Interstand adducts are more cytotoxic with estimates to as few as 20 interstrand crosslinks causing cell death if left unrepaired [14]. While more abundant and repaired slower [15,16], intrastrand lesions are better tolerated via HR and bypass polymerases [17]. Repair of intrastrand crosslinks occurs via the NER pathway [4]. Therefore, while the exact lesion responsible for clinical efficacy remains to be determined, what is clear is that both NER and HR have differential and contributory roles in the cellular sensitivity to cisplatin.

Replication protein A (RPA) is the major human ssDNA binding protein and is required for both NER and HR [18]. The RPA heterotrimer consists of 70 kDa, 32 kDa and 14 kDa subunits with the 70-kDa subunit containing the two major high affinity DNA binding domains (DBD) DBD A and B, as well as DBD C and F. DBD D and E are in the 32-kDa and 14-kDa subunit, respectively. Binding to short stretches of ssDNA (~8–10 nucleotides) is primarily mediated by DBD A and B, while intermediate length ssDNA (~12–23 nucleotides) also involves DBD C. Longer length ssDNA (~28–30 nucleotides) engages DBD D in addition to DBDs A, B and C [19–21]. RPA plays essential and non-redundant roles in both NER and HR, apart from its role in replication and DNA damage checkpoint activation [18]. Each of these roles requires binding of RPA to ssDNA, making RPA–DNA interaction a promising target for anti-cancer therapeutic activity in combination with cisplatin.

Structural analysis of RPA reveals unique protein–DNA interactions that would facilitate the design of potent and selective small molecule inhibitors (SMIs) [22]. It has been also shown that genetic mutants of RPA display defects in DNA repair without impacting DNA replication and vice versa [18,23,24]. This separation of function can be exploited by using chemical probes that exclusively interfere with the DNA repair pathway and that, in conjunction with DNA-damaging agents, would offer a new possibility for cancer treatment. Our group has previously reported both reversible and irreversible chemical inhibitors of RPA [25–28]. The reversible inhibitor TDRL-505 exhibits synergistic effects with DNA damaging agents in a lung cancer cell model. This small molecule hinders the binding of DBD A and B of RPA to ssDNA, which according to *in silico* docking analysis occurs as a consequence of its interaction with DBD B and the DBD A–B interdomain [27]. In the present study we screened a series of TDRL-505 analogs *in vitro* and evaluated their activity in an EOC cell culture model. Structure–activity relationship (SAR) data led us to an enhanced lead compound, TDRL-551. Herein we report the *in vitro*, cellular and *in vivo* activity of the RPA inhibitor TDRL-551 in models of lung and ovarian cancer.

2. Materials and methods

2.1. Protein purification

Full length, heterotrimeric human RPA (fl-RPA) was expressed in *Escherichia coli* and purified by a three column procedure as previously described [29]. The DBD-A/B construct was expressed as a SUMO-His_6-$RPA^{181-432}$ fusion protein. *E. coli* BL21 (DE3) cells in log growth were induced for 3 h with 0.5 mM IPTG at 37 °C. The cells were lysed in buffer containing 50 mM Tris pH 7.5, 300 mM NaCl, 10% sucrose, 10 mM imidazole, 25 μg/mL lysozyme, 1 μg/mL leupeptin, 1 μg/mL pepstatin and 0.5 mM PMSF. The lysate was loaded onto a Ni-NTA column washed and then incubated overnight with wash buffer containing 3 μg/mL ULP1 protease to cleave the SUMO tag. The cleaved His_6-$RPA^{181-432}$ was eluted from the Ni-NTA column with elution buffer containing 350 mM imidazole. The His6-RPA was then further purified on a size exclusion column (SEC) to remove the cleaved SUMO tag fragment. The SEC pool was then concentrated and stored at −80 °C.

2.2. Electrophoretic mobility shift assays (EMSA)

EMSA reactions (20 μL) were performed with 50 nM fl-RPA and 2.5 nM 5′[^{32}P]-labeled 34-base DNA in buffer containing 20 mM HEPES (pH 7.0), 1 mM DTT, 0.001% NP-40, 100 mM NaCl, 5 mM $MgCl_2$ and 50 μg/mL bovine serum albumin (BSA). Chemical compounds, either purchased from ChemDiv or synthesized in our laboratory, were suspended in DMSO and titrated as detailed in each figure. The DMSO concentration in the reaction mixture was kept constant at or below 5%. RPA was incubated with inhibitor or DMSO control in reaction buffer for 30 min before the addition of DNA. Reactions were incubated for 5 min at room temperature and products separated via 6% native polyacrylamide gel electrophoresis. The bound and unbound fractions were then quantified by phosphor-imager analysis using ImageQuant software (Molecular Dynamics, CA) and IC_{50} values calculated by non-linear regression using SigmPlot (Sysat). For EMSA reactions with RPA DBD-A/B, 150 nM DBD-A/B was used and electrophoresis was performed at 4 °C. All other conditions were identical to those described for the full length RPA.

2.3. Chemical synthesis

2.3.1. General

All solvents and chemicals were used as purchased from commercial suppliers. ^{1}H NMR spectra were obtained on a Bruker Avance III 500 MHz NMR spectrometer. Chemical shifts are expressed in parts per million (ppm, δ), relative to tetramethylsilane (TMS) as internal reference. Signals are described as *s* (singlet), *d* (doublet), *dd* (doublet of doublets), *dt* (doubles of triplets), *t* (triplet), *q* (quartet), or *p* (pentet).

2.3.2. 2-chloro-7-ethoxy-3-(3-(4-iodophenyl)-4,5-dihydro-1H-pyrazol-5-yl)quinoline (**7a**)

NaOH (0.83 mL, 2.5 M in water, 2.07 mmol) was added dropwise to a solution of 4-iodoacetophenone (0.36 g, 1.47 mmol) and 2-chloro-7-ethoxyquinoline-3-carbaldehyde (0.35 g, 1.47 mmol) in EtOH (12 mL). After stirring for a 45 min at 40 °C, the reaction mixture was quenched with HCl (1.38 mL, 3 M). The crude mixture containing the resulting enone was then filtered, thoroughly washed with EtOH, and used in the next step without further purification. Hydrazine monohydrate (0.36 mL, 7.33 mmol) was added dropwise to a suspension of the enone obtained in the previous step in EtOH (30 mL). The mixture was refluxed for 1.5 h with stirring, after which it was allowed to cool to room temperature. The obtained solid was filtered and washed with EtOH. Further purification by trituration with EtOH furnished the title compound as an off-white solid (0.57 g, 81% over two steps). ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.41 (*t*, *J* = 7.0 Hz, 3H), 2.89 (*dd*, *J* = 16.5, 10.0 Hz, 1H), 3.67 (*dd*, *J* = 16.5, 11.0 Hz, 1H), 4.20 (*q*, *J* = 7.0 Hz, 2H), 5.19 (*dt*, *J* = 10.5, 3.5 Hz, 1H), 7.27 (*dd*, *J* = 9.0, 2.5 Hz, 1H), 7.34 (*d*, *J* = 2.5 Hz, 1H), 7.44 (*d*, *J* = 8.5 Hz, 2H), 7.74 (*d*, *J* = 8.5 Hz, 2H), 7.84 (*d*, *J* = 3.5 Hz, 1H), 7.97 (*d*, *J* = 9.0 Hz, 1H), 8.42 (*s*, 1H).

2.3.3. 4-(5-(2-chloro-7-ethoxyquinolin-3-yl)-3-(4-iodophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-4-oxobutanoic acid (**9a** or **TDRL-551**)

A round-bottom flask coupled with a reflux condenser and containing a dry mixture of 2-chloro-7-ethoxy-3-(3-(4-iodophenyl)-4,5-dihydro-1H-pyrazol-5-yl)quinoline (**7a**) (0.6 g, 1.25 mmol) and glutaric anhydride (0.14 g, 1.25 mmol) was immersed into a preheated oil bath (65 °C). $CHCl_3$ (24 mL) was then added through the condenser in one portion. The resulting solution was refluxed for 1.5 h with stirring, after which it was allowed to cool to room temperature. The obtained solid was filtered and washed with ethyl acetate. Further purification by trituration with ethyl acetate yielded acid **9a** as an off-white solid (0.53 g, 72%). ^{1}H NMR