DNA Repair 45 (2016) 44–55

Contents lists available at ScienceDirect

DNA Repair

journal homepage: www.elsevier.com/locate/dnarepair

ABT-888 and quinacrine induced apoptosis in metastatic breast cancer stem cells by inhibiting base excision repair via adenomatous polyposis coli

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ARTICLE INFO

Article history:
Received 3 May 2016
Received in revised form 23 May 2016
Accepted 27 May 2016
Available online 3 June 2016

Keywords:
Cancer stem cells
PARP inhibitor
ABT-888
Quinacrine
APC

ABSTRACT

PARP inhibitors in combination with other agents are in clinical trial against cancer, but its effect on cancer stem cells (CSCs) is limited. CSCs are responsible for drug resistance, metastasis and cancer relapse due to high DNA repair capacity. Here, we present preclinical effects of Quinacrine (QC) with ABT-888, a PARP inhibitor, on highly metastatic breast cancer stem cells (mBCSCs). An increased level of Adenomatous polyposis coli (APC) was noted after treatment with ABT-888 in QC pre-treated mBCSCs cells. Increased APC physically interacts with PARP-1 and inhibits PARylation causing the non assembly of base excision repair (BER) multiprotein complex, resulting in an irreparable DNA damage and subsequent apoptosis. Knockdown of APC in mBCSCs inhibited DNA damage, increased BER and PARylation, reduces apoptosis while the over-expression of APC in BT20 (APC low expressing) cells reversed the effect. Thus, combination of QC and ABT-888 decreased mBCSCs growth by activating APC and inhibiting BER within the cells.

1. Introduction

DNA is a storehouse of genetic information and it continuously undergoes damaging alterations under the influence of environmental conditions, accumulation of toxic metabolic byproducts and mistaken DNA replication. To restore the normal base pair sequence and to maintain the genomic integrity, several DNA repair mechanisms have evolved [1]. Base excision Repair (BER), Nucleotide excision repair (NER) and mismatch repair (MMR) constitute the single strand break repair mechanism while homologous recombination (HR) and non-homologous end joining (NHEJ) represents double strand break repair mechanism [2]. Upon DNA damage, poly (ADP) ribose polymerase 1 (PARP-1), which is normally present in the nucleus gets activated and is recruited at the site of DNA damage as a homodimer in a very fast reaction amplified 10–500 fold with the formation of poly (ADP) ribose polymers (PAR) within 15–30 s [2]. The growing PAR chain imparts an overall negative charge. The widespread branching system of PAR on PARP-1 acts as a sign for drawing in and assisting the assembly of a multiprotein complex concerned with chromatin remodeling and DNA repair pathways. Histones (H1 and H2B) are important substrates of PARP-1, which, when, relocated by ADP-ribosylation, facilitates improved accessibility of large multiprotein complex assembled during DNA repair. DNA repair enzymes like DNA ligase III, DNA Pol-β, XRCC-1 are then recruited directly by automodified PARP-1 [3]. PARP-1 is expressed in all the eukaryotic cells [4], however its expression is up-regulated in highly proliferating cells [5]. PARG (Poly- (ADP-ribose) glycohydrolase) leads to PAR hydrolysis and limits PARylation. The balance between PARP-1 and PARG determines the amount of PAR present in the cell [2]. Many researchers across the globe have reported higher expression level of PARP-1 in solid tumors and haematological malignancies [6–8]. PARP inhibitors selectively target the highly proliferating cells due to the difference in the expression level of PARP in normal versus tumor cells. As PARP is a major protein involved in BER, PARP inhibition sensitizes the tumor cells to cytotoxic agents causing DNA damage, that would otherwise be repaired by BER cascade involving DNA glycosylase, AP endonuclease, DNA polymerases, WRN, FEN-1 and PARP-1 [9].

PARP inhibitors in combination with well known DNA damaging agents such as temozolomide and camptothecin, are under clinical trials [10]. Tentori et al. reported the potentiality of PARP inhibitors to distinguish between normal cells and cancer cells and advocated the significant advantage of veliparib (ABT-888) over other well known PARP inhibitors [11]. ABT-888 is a potent PARP-1 and PARP-2 inhibitor. It has also been reported to potentiate the DNA damaging efficacy of temozolomide, platinum agents and cyclophosphamides [9]. Boerner et al. reported enhanced DNA

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http://dx.doi.org/10.1016/j.dnarep.2016.05.034
1568-7864/

damage and cell death by combining PARP inhibitor, ABT-888 with DNA damaging topoisomerase I inhibitor, (Camptothecin 11) CPT11 in preclinical studies [12]. The chemosynthetic DNA damaging agents has some disadvantages, like the non specificity of these agents and the toxicity it imparts towards the normal cells, so, there is a need of the time to look for natural bioactive agents with DNA damaging potentiality.

Quinacrine (QC) is a bioactive small molecule derivative of 9 aminoacridine (9-AA) exhibiting anticancer potentiality against many cancers like colon, breast, pancreas, lung and renal cell carcinoma [13–15]. It is in clinical trial stage II against hormone refractory prostrate cancer [13]. We have already reported that the anti-breast cancer potentiality of QC is via induction of DNA damage, inhibition of topoisomerase activity and downregulation of WNT–TCF signaling by inducing APC [15,16]. We and others have also demonstrated that QC induces autophagy alone as well as in combination, in various cancer models [17–19]. We have also shown that induction of autophagy leads to apoptosis in colon cancer cells by p53/p21 dependent pathway [17]. QC strongly binds to DNA and make DNA adducts. We have also reported that QC alone and in combination with Chk1 inhibitor (SB218078) disrupt the BER activity in breast cancer cells [20]. It is reported that DNA adduct are strongly repaired by BER pathway [21].

Adenomatous polyposis coli (APC) is a 312 KDa protein comprising 2843 amino acids present in both the cytoplasm and nucleus [22]. It regulates Wnt-β-catenin signaling, cell-cell adhesion, migration, apoptosis, chromosomal instability, cell cycle control and DNA repair [23–27]. The link between APC and DNA repair is fascinating as we along with others have shown that APC is induced in response to DNA damaging agents [28–30]. APC contains a DRI (DNA repair inhibitory) domain, a PIP like box traversing amino acids 1245–1273 [28]. Via its DRI domain (Gln1256, Ile1259and Tyr1262), APC physically interacts with Pol-β and FEN-1 thereby blocking strand displacement synthesis in BER [30,31].

Reports suggest the presence of a subpopulation of cancer stem cells (CSCs) in tumors possessing self renewal potentiality and are responsible for cancer relapse [32,33]. CSCs play a major role in tumor initiation, progression, invasion, metastasis and drug resistance [34–36]. Higher expression of drug transporters, DNA repair capacity, relative cell cycle quiescence and resistance to apoptosis are the characteristics of CSCs imparting resistance towards conventional chemo and radiotherapy [37,38].

The ability of CSCs to overcome from toxicity of standard chemotherapeutic agents is due to prompt DNA damage sensor as well as hyperactivated DNA damage repair pathways [39]. Therefore, it would be a great approach to target the DNA damage response (DDR) of CSCs by increasing DNA damage through inhibition of DNA repair. To address this issue, we have studied the effect of ABT-888 on the QC pre-treated MCF-10A-Tr-P-EMT metastatic breast cancer stem cell model of cigarette smoke transformed breast cancer cells (MCF-10A-Tr) using serum deprivation conditions in liquid overlay method.

Recently, we have established a novel cigarette smoke condensate induced-transformed cell line (MCF-10A-Tr) model that could offer a suitable system to study the mechanism of cellular transformation caused by chemical carcinogens and could help to investigate the mechanism of potential anticancer molecules against aggressive and transformed breast cancer cell types [40]. MCF-10A-Tr cells were developed by repeated and continuous exposure to a single dose of cigarette smoke condensate prepared from commercially available Indian cigarette to spontaneously immortalized transformed breast epithelial cell line, MCF-10A. The cancer initiating cells enriched population (MCF-10A-Tr-P-EMT) was made from MCF-10A-Tr cells.

In the present piece of work, we have shown that ABT-888 enhances the DNA damaging effects of QC in mBCSCs by inhibiting the repair process causing persistent irreparable DNA damage which increased damage-induced APC. We propose that the induced APC inhibits the (PAR)ylation activity of PARP1, thereby inhibiting PAR leading to the non assembly of the BER multiprotein complex and finally causing apoptosis.

2. Materials and methods

2.1. Cell culture and reagents

Breast cancer (MCF-10A-Tr) and BT20 (low or null expression of APC) [29], cells were grown in DMEM supplemented with 10% FBS, 1.5 mM l-glutamine and 1% antibiotic (100 U/ml of penicillin, 10 mg/ml of streptomycin) in a humidified atmosphere of 5% CO_2 at 37 °C. MCF-10A-Tr-P-EMT cells were cultured in DMEM-F12 supplemented with 10% FBS, 1.5 mM l-glutamine and 1% antibiotic (100 U/ml of penicillin, 10 mg/ml of streptomycin) in a humidified atmosphere of 5% CO_2 at 37 °C. The spontaneously immortalized transformed breast epithelial cells (MCF-10A) was grown and cultured in DMEM-F12 containing 10% FBS, 1% antibiotic, 0.5 μg/ml of hydrocortisone, 100 ng/ml of cholera toxin, 10 μg/ml of EGF and 1.5 mM l-glutamine in a 5% CO_2 incubator at 37 °C. Cell culture reagents were procured from HIMEDIA, India. QC (Cat # Q3251), was purchased from Sigma Chemical Co. (St Louis, MO). ABT-888 (ALX-270-444) was purchased from Enzo Life Sciences (Farmingdale, NY, USA) and Rucaparib (AG-014699) was purchased from Selleck Chemical, Houston, TX, USA. Anti-CD-44 (ab23557), anti-CD-24 (ab77219), anti-RAD 51 (ab63801), anti-DNA PKcs (ab70250), anti-Pol-δ (ab96796) and anti-XRCC-1 (ab47920) were purchased from Abcam, MA, USA. Anti-APC (#OP44) was purchased from Calbiochem, CA, USA. Anti-E-CADHERIN (#3195), anti-VIMENTIN (#5741), anti-BAX (#2772), anti-Bcl-xL (#2764), anti-γ-H2AX (#9718P), anti-CASPASE 3 (#9662), anti-Pol-H (#13848), anti-PARP-1 (#9542), anti-WRN (#4666) and anti-FEN-1 (#2746) were purchased from Cell Signaling Technology, MA, USA. GAPDH (sc-25778) is purchased from Santa Cruz Biotechnology Inc., CA, USA. Anti-Pol-β (NB#600-1025) is purchased from Novus Biologicals, CO, USA. Anti-CD-133 is purchased from MACS, Miltenyl Biotec Asia Pacific Pte Ltd. Anti-PAR (4335-MC-100) antibody was purchased from Trevigen, Gaithersburg, MD. Anti-CXCR4 (SAB3500383) was purchased from Sigma Chemical Co. (St Louis, MO). In combination treatment, cells were first exposed to 5 μM of QC for 2 h and medium was aspirated and then 10 μM of ABT-888 was added and allowed the cells to grow for another 24 h.

2.2. Development of the metastasis model

Metastasis model of MCF-10A-Tr was established according to the protocol (Liquid overlay method) referred earlier with minor modifications [41–44]. In brief, 0.5% agarose in Milli-Q water was coated onto a 12 well plate and allowed to air-dry under aseptic conditions. Approximately, 4×10^4 MCF-10A-Tr cells in serum free medium were overlaid onto the agarose coated wells and incubated 3–4 days for the formation of spheroids. Uniform mammospheres were observed after 6–7 days of incubation which were termed as MCF-10A-Tr-MAMMO. MCF-10A-Tr-MAMMO was isolated by centrifugation at 850 rpm for 5 min followed by dissociation using trypsin-EDTA. The dissociated single cells of MCF-10A-Tr-MAMMO were resuspended in 10% serum containing (10% FBS containing) DMEM-F12. Viable floating spheroids regained the property of adherence with the availability of serum and formed a monolayer which was designated as MCF-10A-Tr-P-EMT (MCF-10A-Tr-post epithelial to mesenchymal transformed which were already undergone epithelial to mesenchymal transition). MCF-10A-Tr-P-