Bioorganic & Medicinal Chemistry Letters 21 (2011) 4248–4251

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

A transcription factor hijacking to regulate RARα by using a chimeric molecule of retinoic acid and a DNA alkylator

Kyung Hoon Min [a], Byoung-Gu Yun [b], Yoonsung Lee [b], Jiho Song [a], Won-Hoon Ham [b], YoungJoo Lee [c], Hyun-Ju Park [b,*]

[a] College of Pharmacy, Chung-Ang University, Seoul 156-756, Republic of Korea
[b] School of Pharmacy, Sungkyunkwan University, Suwon 449-746, Republic of Korea
[c] Department of Bioscience & Biotechnology, College of Life Science, Sejong University, Seoul 143-747, Republic of Korea

ARTICLE INFO

Article history:
Received 12 January 2011
Revised 9 May 2011
Accepted 19 May 2011
Available online 25 May 2011

Keywords:
Transcription factor hijacking
Chimeric molecule
Chlorambucil
All-*trans* retinoic acid
Retinoic acid receptor α

ABSTRACT

As a model compound for the transcription factor hijacking mechanism of action of DNA damaging agent that simultaneously bind to the nuclear receptor, we designed and synthesized a chimeric molecule, RA-mustard, which can bind with both retinoic acid receptor α (RARα) and DNA. The interaction between RA-mustard with RARα was confirmed by binding assay using RARα-overexpressing cell extract. RA-mustard-modified DNA diminished the RARα-dependent luciferase expression in the RARα-abundant cells.

The development of small molecule regulators has attracted considerable attention in the context of expanding our understanding of complicated biological processes. Chemical genetic approaches have proven to be extremely useful in studies of protein function without any genetic manipulation. The design and development of small molecules capable of inducing the selective loss of a targeted protein is one of the major goals of chemical genetics. Meanwhile, the design and development of chimeric small molecules with two functional moieties capable of interacting simultaneously with two molecular targets is also receiving great attention.[1] Several chimeric small molecules that regulate biological events have been reported. STF1 (a hairpin polyamide-wrenchnolol)[2] and FkCsA (FK506-cyclosporin A)[3] were designed as transcription activators, whereas PROTACs[4] were designed to degrade target proteins.

Cisplatin reacts with DNA to form 1,2-intrastrand cross-links, and it is generally accepted to be responsible for its anticancer activity. Interestingly, the ribosomal RNA transcription factor hUBF binds preferentially to cisplatin-DNA adducts, thus reducing the hUBF binding to its native DNA regulatory site and abolishing hUBF-dependent transcription. It is evident that this hijacking of a transcription factor could lead to functional inhibition.[5] Based on this notion, bifunctional ligands that alkylate DNA and bind to specific nuclear transcription factors have been designed. Examples of such ligands include estradiol-linked genotoxicants[6,7] and 11β-dichloro,[8,9] which disrupt gene expression by hijacking estrogen receptor (ER) and androgen receptor (AR), respectively. These activities eventually manifest as selective toxicity against cancer cells over-expressing corresponding nuclear receptors, ER-positive MCF[6,7] and AR-positive LNCaP[8,9] cells.

Our research interests include the design and development of chimeric small molecules that regulate proteins of interest, and verifying the transcription factor hijacking model. We chose to target the retinoic acid receptor (RARα), a nuclear receptor

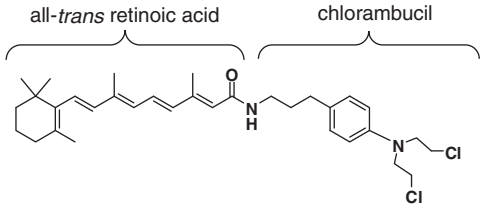


Figure 1. Structure of RA-mustard, a simple chimeric molecule that consists of an all *trans*-retinoic acid moiety for RARα and chlorambucil for DNA alkylation.

* Corresponding author.
E-mail address: hyunju85@skku.edu (H.-J. Park).

0960-894X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2011.05.062

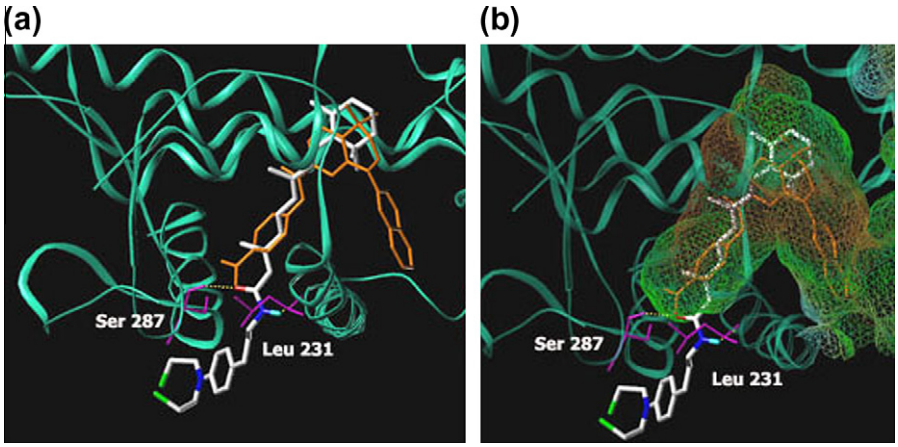
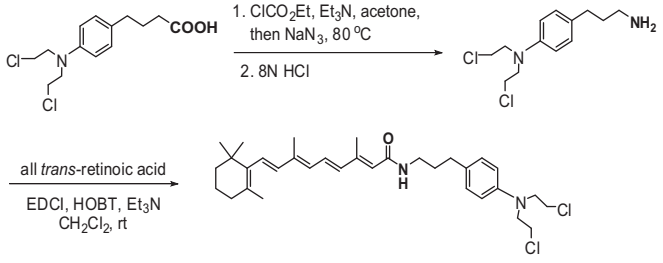


Figure 2. (a) A docking model of RA-mustard in the ligand-binding site of RARα. Bound antagonist BMS614 (orange) in the X-ray structure (PDB id: 1DKF) is shown for comparison. The retinoic acid moiety of RA-mustard occupies the binding site of BMS614, whereas its nitrogen mustard moiety is exposed outside the binding pocket. (b) Lipophilic potential surface map of the ligand-binding pocket of RARα is displayed in the docking model. Lipophilicity increases from blue (hydrophilic) to brown (lipophilic).



Scheme 1. Synthesis of RA-mustard.

transcription factor, which is an attractive target because it plays essential roles in cell proliferation and differentiation.[10,11]

The present study, in which a hybrid molecule of an endogenous ligand (retinoic acid) and a DNA alkylating agent (chlorambucil) is used as a probe molecule, aims to demonstrate that a transcription factor hijacking mechanism works in the RARα-abundant cells.

As an initial step, we designed a simple chimeric molecule (RA-mustard) to hijack RARα (Fig. 1). All *trans*-retinoic acid (ATRA) is an endogenous ligand for RARα, and nitrogen mustard chlorambucil can form a covalent bond with DNA. ATRA (a RARα binding moiety) and nitrogen mustard (a DNA binding moiety) are linked to form a hetero-bifunctional molecule. We expected that RA-mustard would inhibit the function of RARα by driving RARα to ligand-modified DNA sites rather than to specific promoter sequences.

RA-mustard was subjected to docking analysis to determine whether it can access the binding site of RARα. RA-mustard was docked into the X-ray structure of RARα (PDB ID: 1DKF)[12] using the FlexX program. The docking model showed that the retinoic acid moiety can access the binding pocket for antagonist (BMS-614), whereas the nitrogen mustard moiety protrudes outside the binding site and can interact with DNA (Fig. 2). Therefore, we synthesized the chimeric molecule, RA-mustard (Scheme 1). Chlorambucil was readily converted to an amine using the Curtis rearrangement. EDCI-mediated coupling of the amine with all *trans*-retinoic acid gave RA-mustard (see Supplementary data for detailed information about syntheses and spectroscopic characterization of the compounds).

We carried out the competitive binding assay in which RA-mustard competes for [^{3}H]-labeled ATRA in the reaction with RARα over-expressing COS cell extract. RA-mustard inhibited the binding of ATRA to RARα in a dose-dependent manner (see Supplementary data).

Next, we explored whether the DNA adduct of RA-mustard could be formed. Accordingly, RA-mustard was incubated with [γ-^{32}P]-5′-end labeled duplex oligonucleotide DNA. As shown in Figure 3a, RA-mustard formed cross-linked and monoalkylated DNA adducts, indicating that RA-mustard maintains the ability to alkylate DNA. The major alkylation site of DNA by chlorambucil is the N^7 positions of guanine base.[13] The DNA alkylation sites were confirmed by strand breakage assays, in which piperidine treatment generates strand breaks at the drug-modified sites.[13] As compared with chlorambucil, the sequence selectivity of RA-mustard alkylation was also unchanged (Fig. 3b).

To verify the interaction between RARα and RA-mustard modified DNA, electrophoretic mobility shift assay (EMSA)[14] was conducted (Fig. 4). The reaction product obtained from the binding reaction of RARα-overexpressed cell extract with RA-mustard/DNA adduct migrated more slowly than the corresponding RARα-free RA-mustard/DNA adduct, whereas no shifted band was observed with the chlorambucil/DNA adduct, indicating that the RA-mustard/DNA adduct can hijack RARα.

Taken together, these findings demonstrate that RA-mustard is a bifunctional agent that interacts with both RARα and guanine bases in duplex DNA.

The ability of RA-mustard to abolish the transcriptional activity of RARα by hijacking was evaluated in COS cells using a reporter construct in which transcription of the reporter gene was controlled by retinoic acid response element (RARE) (Fig. 5a).[15] ATRA responsive luciferase reporter gene (RARE-luc) was found to be activated by ATRA in the presence of RARα. Cotransfection of RA-mustard modified plasmid DNA into COS cells with RARE-luc and plasmid expressing RARα was found to suppress the transcription of the reporter gene in a dose-dependent manner (Fig. 5b).

In summary, we describe a transcription factor hijack system based on the use of a chimeric small molecule that interacts with both DNA and RARα (a nuclear receptor transcription factor). The design of this compound by molecular modeling resulted in the development of a bifunctional agent retaining both good affinity for RARα and the capacity to covalently modify DNA. We found that the hybrid molecule forms cross-linked DNA adducts and hijacks RARα, and that this potentially leads to the specific inhibition of RARα-dependent gene expression. We believe that the nitrogen mustard moiety could be replaced with other useful functional molecules, such as, sequence specific DNA binder polyamides or

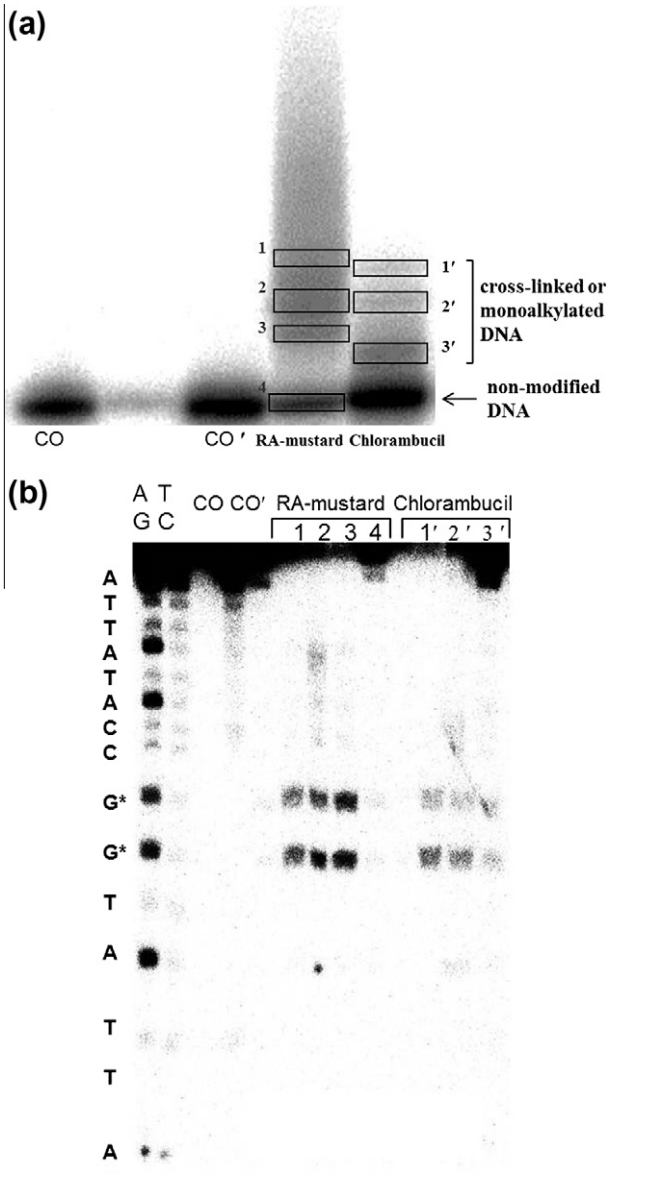


Figure 3. DNA Alkylation with RA-mustard. (a)Autoradiogram of a 20% denaturing polyacrylamide gel showing the drug-modified and non-modified species. After the reaction of 5′-end-labeled 16-base-pair duplex oligomers ([γ-^{32}P]-5′-AATTATGGC-CATATTA-3′/5′-TAATATGGCCATAATT-3′) with RA-mustard and chlorambucil, respectively. CO is without drug treatment and CO′ is from the reaction with DMSO. The numbered bands were excised and the DNA was extracted by 10% annealing buffer. (b)Autoradiogram of 12% denaturing polyacrylamide gels showing the DNA strand-breakage patterns of the oligonucleotides (1–4 and 1′–3′) excised from the gel in (a). AG and TC represent the purine and pyrimidine specific chemical cleavage reaction. Asterisks indicate the drug-modified bases.

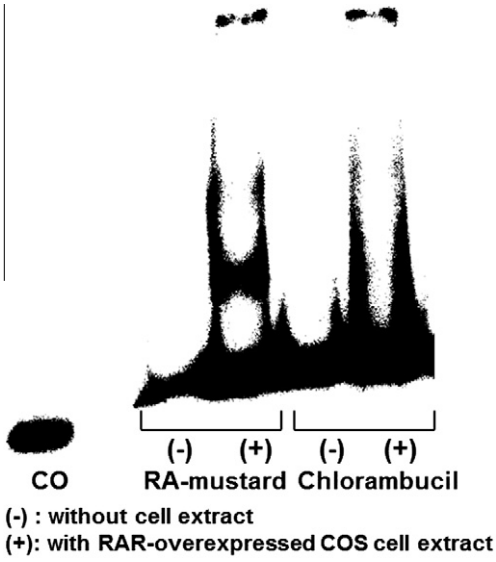


Figure 4. Electrophoretic mobility shift assay.[14] A 5′-end-labeled DNA fragment and RA-mustard complex was incubated with RARα-overexpressing cell extract, and the then reaction mixture was subjected to electrophoresis using a 5% low salt nondenaturing gel.

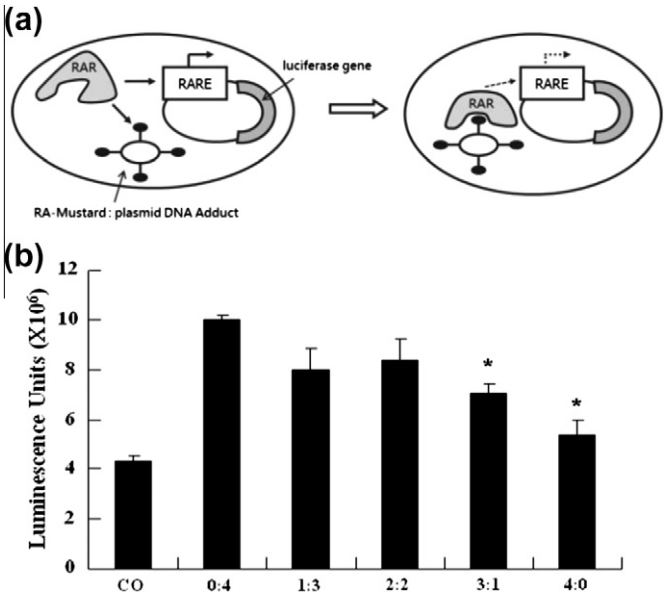


Figure 5. (a) A schematic of the ATRA responsive reporter gene assay. RA-mustard modified plasmid DNA was co-transfected with an ATRA-responsive luciferase reporter gene (RARE-luc reporter plasmid) into COS cells. (b) Mixtures of various ratios of RA-mustard modified to non-modified plasmid DNA were then co-transfected with RAR vector and RARE-Luc reporter plasmid into COS cell. All cells were pre-treated with ATRA except for CO (control). After incubation for 24 h, luciferase activities were measured.[15] *p <0.05 versus the sample with 0:4.

other alkylating agents. Furthermore, we believe that our results provide potentially useful information for the design of programmable transcription inactivator.

Acknowledgments

This study was supported by a Grant of the Korea Healthcare technology R&D Project, Ministry for Health & Welfare, Republic of Korea (Grant A092006), and in part by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (Grants 2010-0029358 and 314-2008-1-E00305).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.05.062.

References and notes

1. Corson, T. W.; Aberle, N.; Crews, C. M. *ACS Chem. Biol.* **2008**, *3*, 677.
2. Jung, D.; Choi, Y.; Uesugi, M. *Drug Discovery Today* **2006**, *11*, 452.
3. Belshaw, P. J.; Ho, S. N.; Crabtree, G. R.; Schreiber, S. L. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 4604.

4. Sakamoto, K. M.; Kim, K. B.; Kumagai, A.; Mercurio, F.; Crews, C. M.; Deshaies, R. J. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 8554.
5. Treiber, D. K.; Zhai, X.; Jantzen, H.-M.; Essigmann, J. M. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 5672.
6. Sharma, U.; Marquis, J. C.; Dinaut, A. N.; Hiller, S. M.; Fedeles, B.; Rye, P. T.; Essigmann, J. M.; Croy, R. G. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3829.
7. Kim, E.; Rye, P. T.; Essigmann, J. M.; Croy, R. G. *J. Inorg. Biochem.* **2009**, *103*, 256.
8. Marquis, J. C.; Hiller, S. M.; Dinaut, A. N.; Rodrigues, D.; Mitra, K.; Essigmann, J. M.; Croy, R. G. *Chem. Biol.* **2005**, *12*, 779.
9. Hiller, S. M.; Marquis, J. C.; Zayas, B.; Wishnok, J. S.; Liberman, R. G.; Skipper, P. L.; Tannenbaum, S. R.; Essigmann, J. M.; Croy, R. G. *Mol. Cancer Ther.* **2006**, *5*, 977.
10. Wu, S.; Zhang, D.; Zhang, Z. P.; Soprano, D. R.; Soprano, K. J. *Oncogene* **1998**, *17*, 2839.
11. Muller, P.; Kietz, S.; Gustafsson, J. A.; Strom, A. *J. Biol. Chem.* **2002**, *277*, 28376.
12. Bourguet, W.; Vivat, V.; Wurtz, J.-M.; Chambon, P.; Gronemeyer, H.; Moras, D. *Mol. Cell* **2000**, *5*, 289.
13. Bauer, G. B.; Povirk, L. F. *Nucleic Acids Res.* **1997**, *25*, 1211.
14. *Electrophoretic mobility shift assay (EMSA)*. Nuclear extracts were isolated from COS cells overexpressing RARα, according to the manufacturer's instructions (Activemotif, USA). Protein concentrations were determined using the Bradford method. Gel retardation assays were performed as reported. Probe DNA (2000–5000 cpm/μl) from [γ-^{32}P]-5′-end-labeled plasmid (pUC18) fragmented with restriction enzyme EarI and HindIII, 10 mM RA-mustard or 1 mM chlorambucil were mixed in 10% annealing buffer (1 mM Tris–HCl, 10 mM NaCl) and were incubated at 37 °C for 30 min. DNA fragment cross-linked with RA-mustard was reacted with RARα overexpressing cell extract in DNA binding buffer (15 mM Tris pH7.9, 80 mM KCl, 4 mM DTT, 0.2 mM EDTA, 10% glycerol) containing 10 μg BSA and 10 mg/ml p(dIdC) carrier DNA at room temperature for 20 min. Reaction mixtures were loaded onto 5% low salt gels were dried for 40 min and exposed to a molecular imaging plate. Images were recorded using a BAS-250 imager.
15. *Transient transfection and luciferase assays*. COS cells were seeded in 24-well plates at a density of 7×10^4 cells/well. After 24 h, plasmids were transiently transfected into cells using a calcium phosphate-DNA coprecipitation method. A total of 0.5 μg of DNA in 25 μl of $CaCl_2 \cdot H_2O$ (250 mM $CaCl_2$) was mixed with 25 μl of 2× HBS (280 mM NaCl, 10 mM KCl, 1.5 mM $Na_2HPO_4 \cdot 2H_2O$, 12 mM dextrose, 50 mM HEPES) with constant bubbling and within 5–10 min this solution was added to each well. The next day, transfected cells were washed with PBS and add enough 1X lysis buffer to cover the cells. Cell lysate was stored at −70 °C. Luciferase activities were determined using the luciferase assay system using 20 μl of cell lysate with 100 μl of Luciferase Assay Reagent (Promega, Madison, WI) and an AutoLumat LB9507 luminometer. Results are expressed in relative light units. The means and standard deviations of triplicate samples are shown. All transfection experiments were repeated three or more times and similar results were obtained. Statistical analyses were performed by Student's *t*-test for luciferase assays using the SPSS 12.0 statistical software program (SPSS, Chicago, IL). The results were considered to be statistically significant at $p < 0.05$.