The antitumor drug candidate 2-(4-amino-3-methylphenyl)-5-fluorobenzothiazole induces NF-κB activity in drug-sensitive MCF-7 cells

Eileen Brantley[a], Vyomesh Patel[b], Sherman F. Stinson[a], Valentina Trapani[c], Curtis D. Hose[d], Henry P. Ciolino[e], Grace C. Yeh[e], J. Silvio Gutkind[b], Edward A. Sausville[f] and Andrea I. Loaiza-Pérez[f]

2-(4-Amino-3-methylphenyl)-5-fluoro-benzothiazole (5F 203) potently inhibits MCF-7 breast cancer cell growth in part by activating the aryl hydrocarbon receptor (AhR) signaling pathway. Ligands for the AhR (i.e. dioxin) have also been shown to modulate the NF-κB signaling cascade, affecting physiological processes such as cellular immunity, inflammation, proliferation and survival. The objective of this study was to investigate the effect of 5F 203 treatment on the NF-κB signaling pathway in breast cancer cells. Exposure of MCF-7 cells to 5F 203 increased protein–DNA complex formation on the NF-κB-responsive element as determined by electrophoretic mobility shift assay, but this effect was eliminated in MDA-MB-435 cells, which are resistant to the antiproliferative effects of 5F 203. An increase in NF-κB-dependent transcriptional activity was confirmed by a significant increase in NF-κB-dependent reporter activity in sensitive MCF-7 cells, which was absent in resistant MDA-MB-435 cells and AhR-deficient subclones of MCF-7 cells. Inhibition of NF-κB activation enhanced the increase in xenobiotic response element-dependent reporter activity in MCF-7 cells when treated with 5F 203. The drug candidate 5F 203 also induced mRNA levels of IL-6, an NF-κB-responsive gene, in MCF-7 cells, but not in MDA-MB-435 cells, as determined by quantitative RT-PCR. These findings suggest that 5F 203 activation of the NF-κB signaling cascade may contribute to 5F 203-mediated anticancer activity in human breast cancer MCF-7 cells. *Anti-Cancer Drugs* 16:137–143
© 2005 Lippincott Williams & Wilkins.

Anti-Cancer Drugs 2005, **16**:137–143

Keywords: 5F 203, aryl hydrocarbon receptor, breast cancer, interleukin-6, NF-κB

[a]Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute–Frederick, NIH, Frederick, MD, USA, [b]Oral and Pharyngeal Cancer Branch, National Institute of Dental and Craniofacial Research, Bethesda, MD, USA, [c]Cancer Research Laboratories, School of Pharmaceutical Sciences, University of Nottingham, Nottingham, UK, [d]SAIC–Frederick, National Cancer Institute at Frederick, Developmental Therapeutics Program, STB–Functional Genomics Laboratory, Frederick, MD, USA, [e]Cellular Defense and Carcinogenesis Section, Laboratory of Metabolism, Center for Cancer Research, National Cancer Institute–Frederick, NIH, Frederick, MD, USA and [f]Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, Bethesda, MD, USA.

Sponsorship: This work was supported from intramural NCI resources.

Correspondence to E. A. Sausville, University of Maryland Greenebaum Cancer Center, 22 S. Greene Street, Baltimore, MD 21201-1595, USA.
Tel: +410 328 7394; fax: +410 328-6896;
e-mail: esausville@umm.edu

Received 12 October 2004 **Accepted** 9 November 2004

Introduction

The anticancer drug candidate 2-(4-amino-3-methylphenyl)-5-fluorobenothiazole (5F 203, NSC 703786) displays potent and selective anticancer activity in *in vitro* and *in vivo* models of breast, renal and ovarian cancers similar to the parent, unfluorinated benzothiazole [1]. This structurally simple compound contains a benzene ring fused to a thiazole ring [2]. Fluorination in the 5-position of the benzothiazole pharmacophore has achieved sustained, potent antiproliferative activity, unlike the unfluorinated benzothiazole, which tended to demonstrate reduced antiproliferative efficacy at higher concentrations [3]. Due to these favorable observations, a lysyl amide prodrug form of 5F 203 has been scheduled to enter phase I clinical trials in 2004 (Fig. 1).

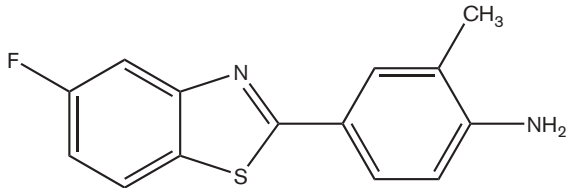
Previous investigations have demonstrated that the antitumor activity of 5F 203 is dependent on its ability to activate the aryl hydrocarbon receptor (AhR) [4]. The AhR is a cytosolic protein that, when activated by a ligand, translocates to the nucleus and binds to a protein partner, the aryl hydrocarbon nuclear translocator (ARNT). Together these proteins form a transcription factor that binds to the xenobiotic response element (XRE) present in the promoter region of the *CYP1A1* gene, inducing its transcription. *CYP1A1* encodes the enzyme cytochrome P-450 1A1 (CYP1A1). Studies have shown that the antitumor activity of 5F 203 results from CYP1A1 enzyme activity, generating metabolites which induce DNA adduct formation, cell cycle arrest and apoptosis in sensitive tumor cells [4,5]. Thus, 5F 203 triggers its own activation to antitumor metabolites once it activates the AhR signaling pathway. The absence of a functional AhR renders cells resistant to 5F 203.

The NF-κB signaling pathway plays a critical role in the regulation of apoptosis and cell cycle progression [6–8].

0959-4973 © 2005 Lippincott Williams & Wilkins

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

Fig. 1



Structure of the candidate anticancer agent 2-(4-amino-3-methylphenyl)-5-fluorobenzothiazole (5F 203, NSC 703786).

Inactive cytosolic NF-κB remains bound to IκBα. Activation of the components of the NF-κB signaling group results in phosphorylation of IκBα, which is rapidly degraded, allowing NF-κB to translocate to the nucleus, where it binds to consensus sites in the promoter region of target genes which promote cell survival [9]. Certain anti-cancer agents exert their desired effect in part by inhibiting the NF-κB pathway [7,10].

Ligands of the AhR, such as the xenobiotic dioxin, have been shown to activate NF-κB signaling [11]. A previous study demonstrated an interaction between the RelA NF-κB subunit and the AhR, suggesting that cross-talk between the NF-κB and AhR pathways occurs [12]. Since 5F 203 activates the AhR, we investigated its effect on NF-κB signaling. To examine this, we used 5F 203-sensitive MCF-7 human breast cancer cells, and two resistant cell lines, AH^{R100}, an AhR-deficient variant of MCF-7 cells [13], and MDA-MB-435 human breast cancer cells. The latter two cell lines possess a defective AhR-signaling pathway and lack *CYP1A1* inducability following 5F 203 treatment [4,14,15]. We demonstrate, for the first time, that 5F 203 activates NF-κB signaling in drug-sensitive cells, but not in resistant cells.

Materials and methods

Drug treatment and cell culture

5F 203 was synthesized in the laboratory of [3] and originally obtained from Professor Malcolm Stevens (University of Nottingham). This benzothiazole was dissolved in 100% DMSO to make a stock solution of 10 mM which was stored in aliquots at –20°C. Just prior to experimental work, the stock solution was diluted in culture medium to obtain the desired concentrations (0.001–100 μM) and cells were exposed for specified time points. Control samples contained those cells treated with vehicle only (0.1% DMSO in medium). Tumor necrosis factor (TNF)-α (Invitrogen, Carlsbad, CA) served as a positive control (10 ng/ml). MCF-7 and MDA-MB-435 cancer cell lines were obtained from the National Cancer Institute Repository at the National Cancer Institute (Frederick, MD). AhR-deficient (AH^{R100}) cells were generated from wild-type MCF-7 cells after continuous exposure to benzo[*a*]pyrene resulting in a 100-fold higher resistance to benzo[*a*]pyrene and reduced amounts of AhR as described previously [13]. All cell lines were maintained in RPMI 1640 medium (Quality Biological, Gaithersburg, MD) containing 10% fetal bovine serum (Hyclone, Logan, UT) in a 37°C incubator with 5% CO_2.

Transfections and luciferase assay

To measure NF-κB activation, MCF-7, AH^{R100} and MDA-MB-435 cells were plated at a density of 2×10^5 cells/well in six-well plates and allowed to adhere overnight (16 h) after which they were co-transfected for 3 h with 0.1 μg. of NF-κB expression vector pNF-κB luc (Stratagene, La Jolla, CA), 0.1 μg of empty vector for pNF-κB-luc, which served as a negative control, in the presence or absence of 0.15 μg of h-IκBα dominant negative (MAD3) cloned in the expression vector pMT 2T [16], which served as an inhibitor of NF-κB, using Lipofectamine (Invitrogen Life Technologies, Carlsbad, CA). Transfected cells were allowed to recover for 24 h in medium containing 10% FBS before being treated with 10 nM TNF-α (3 h), 1 μM 5F 203 (8 h) or 0.1% DMSO (vehicle control). The 1 μM concentration was used since this represented a concentration within the therapeutic range (0.01–2.0 μM) for 5F 203 which caused maximal CYP1A1 induction.

To measure XRE activation, cells were co-transfected for 3 h with 1.5 μg of pTX.Dir (XRE reporter plasmid) in the presence or absence of 0.15 μg of pMT 2T plasmid using Lipofectamine (Invitrogen) in serum-free medium. Transfected cells were allowed to recover for 24 h in medium containing 10% FBS before being treated with 5F 203 (9 h) or 0.1% DMSO (vehicle control).

Following treatment of transfected cells from either group, cells were lysed with 1× passive lysis buffer (Promega, Madison, WI) and luciferase activity of lysates was assayed using the Dual-Luciferase Assay System (Promega) following the manufacturer's instructions. Transfection efficiency was monitored by *Renilla reniformis* (1.5 μg) luciferase activity using the pRL-TK plasmid as an internal control [17].

RNA extraction and quantitative real-time RT-PCR (QRT-PCR) analysis

RNA was isolated from MCF-7 (wild-type) and MDA-MB-435 breast carcinoma cell lines treated with 5F 203 (0.01–1.0 μM) or 0.1% DMSO (control) for 12 or 24 h using the RNeasy 96 kit and QIAvac vacuum manifold (Qiagen, Valencia, CA). The concentration and purity of the RNA were determined by measuring the optical densities at 260 and 280 nm. A ratio of $A_{260}/A_{280} > 1.7$ was required for use in these studies.

Interleukin (IL)-6 gene expression was evaluated in RNA extracts using QRT-PCR. RNA was reverse transcribed

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

using a reverse transcription kit (PE Biosystems, Foster City, CA) and 5 ng of the resulting cDNA was used for each PCR reaction. The PCR reactions were measured with the ABI Prism 7700 sequence detection system using SYBR Green master mix reagent kit in 50 µl reactions. Primers and probes IL-6 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), the endogenous control, were designed with Primer Express software (PE Biosystems) using the gene bank sequence for human IL-6 (forward primer 5′-TGCGTCCGTAGTTTCCTTCT-3′ and the reverse primer 5′-GCCTCAGACATCTC-CAGTCC-3′) and GAPDH sequence (forward primer 5′-GACTTCCAAGCTGGCCGTG-3′ and the reverse primer 5′-CTCCTTGGCAAAACTGCACC-3′). Primer concentrations for IL-6 were 300 nM and for GAPDH 100 nM. Thermocycler parameters were 30 min at 48°C, 10 min at 95°C and 40 PCR cycles of 15 s at 95°C and 1 min at 60°C. All RNA samples were tested in triplicate PCR reactions. Data was analyzed using the comparative CT method (Perkin-Elmer *User Bulletin 2*) and induction of IL-6 was expressed relative to untreated control levels. For the endogenous control GADPH, human primers and probes were used (Applied Biosystems, Foster City, CA).

Electrophoretic mobility shift assay (EMSA)

Nuclear extracts were prepared from cells treated with 5F 203, 0.1% DMSO (vehicle control) or TNF-α (positive control) by a previously described method [18]. To determine NF-κB DNA-binding, double-stranded NF-κB binding oligonucleotide (5′-AGTTGAGGGGACTTTCC-CAGG-3′; Santa Cruz Biotechnology, Santa Cruz, CA) containing the consensus binding site for NF-κB (indicated in the underlined portion) was end-labeled with T4 polynucleotide kinase (Amersham Biosciences, Piscataway, NJ) and [γ-^{32}P]ATP. Binding reactions were performed in a 15-µl reaction mixture containing 10 nM Tris–HCl, pH 7.5, 4% glycerol, 1 mM $MgCl_2$, 50 mM NaCl, 0.5 mM EDTA, 0.5 mM DTT, 1 µg poly(dI–dC) (Amersham) and nuclear protein extract (10 µg). Reactions were incubated for 10 min at room temperature. After adding the DNA probe (approximately 100 000 c.p.m./reaction), all samples were incubated for an additional 20 min at room temperature. The resulting DNA–protein complexes were separated from free DNA under non-denaturing conditions on a 6% polyacrylamide gel (Novex, San Diego, CA) under high ionic strength. Gels were dried, imaged by autoradiography, and quantitated using the UVP Bioimaging BioChemi System with UVP EpiChemi II Darkroom and Canon TV camera and zoom lens and LabWorks Software, version 4.0 (UVP, Upland CA).

Data analysis

All values are expressed as mean ± SEM. Statistical comparisons between control versus 5F 203-treated cells were determined using the Student's *t*-test. $p < 0.05$ was considered statistically significant as indicated by an asterisk. All statistical analyses were done using Instat software (GraphPad Software, San Diego, CA).

Results

5F 203 activates NF-κB in MCF-7 cells

Using EMSA, we examined the effect of 5F 203 on the activation of DNA-binding capacity of NF-κB for the NF-κB consensus site. As shown in Fig. 2(A), there was constitutive binding of NF-κB with the consensus sequence in vehicle-treated cells that was increased in cells treated with a known NF-κB activator TNF-α (positive control). In nuclear extracts isolated from MCF-7 cells treated with 5F 203, there was a rapid activation of NF-κB DNA binding, which increased in a time-dependent manner (Fig. 2A). A 2-fold increase was observed as early as 5 min, eventually resulting in a nearly 4-fold increase following a 12 h treatment. Antibodies for NF-κB suppressed this binding which indicated that 5F 203 specifically activated NF-κB (data not shown). Additionally, antibodies for AhR and ARNT also diminished NF-κB binding which suggested that NF-κB and AhR interact in these cells (data not shown). In MDA-MB-435 cells, there was no constitutive NF-κB binding. Binding was increased remarkably in these cells when they were treated with TNF-α; however, there was no appreciable NF-κB binding in these cells following treatment with 5F 203 (Fig. 2B).

5F 203 induces NF-κB-dependent transcriptional activity

We transiently transfected cells with a luciferase reporter vector driven by NF-κB. In MCF-7 cells treated with the positive control, TNF-α, there was an approximately 5-fold increase in NF-κB-dependent transcription (Fig. 3). 5F 203 significantly increased NF-κB-dependent transcription (approximately 3-fold) in MCF-7 cells. In these cells, the increase in NF-κB transcriptional activity was concentration-dependent (data not shown).

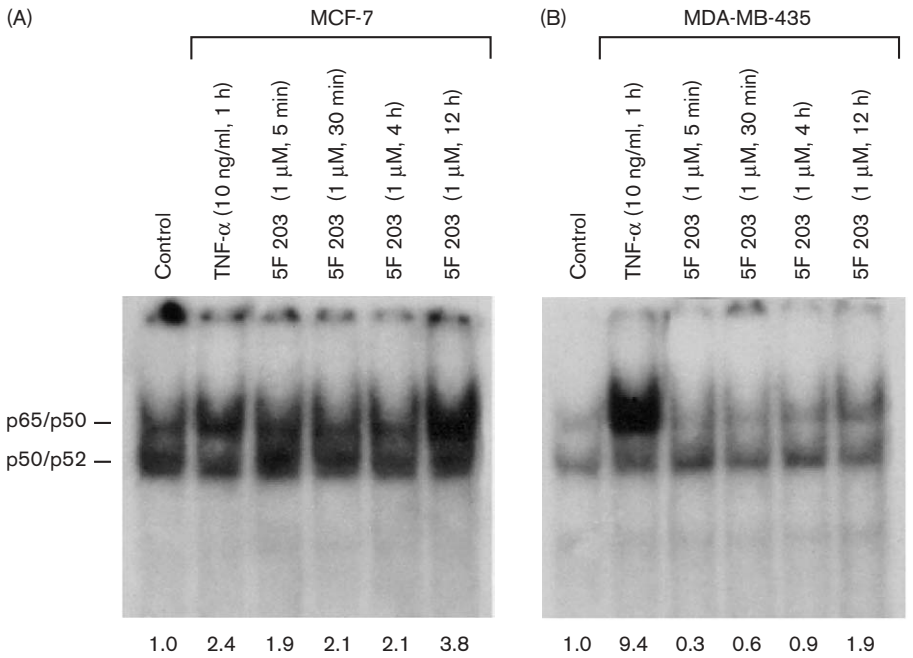
In AH^{R100} and MD-MB-435 cells, 5F 203 failed to induce significant increases in NF-κB-dependent transcription. Co-transfection of all three cell lines with a dominant negative IκB vector, which has been previously shown to effectively inhibit NF-κB [16], suppressed transcription revealing the ability of 5F 203 to specifically activate NF-κB transcription.

Inhibition of NF-κB transcriptional activity enhances XRE-dependent reporter activity in 5F 203-treated MCF-7 cells

As we have previously demonstrated, 5F 203 treatment of MCF-7 cells transfected with an XRE-dependent luciferase reporter vector caused an increase in XRE-dependent transcription [4]. However, co-transfection with the dominant negative NF-κB in cells approximately doubled XRE-dependent transcription in these cells (Fig. 4). There was no appreciable XRE-dependent transcription detected in MDA-MB-435 cells.

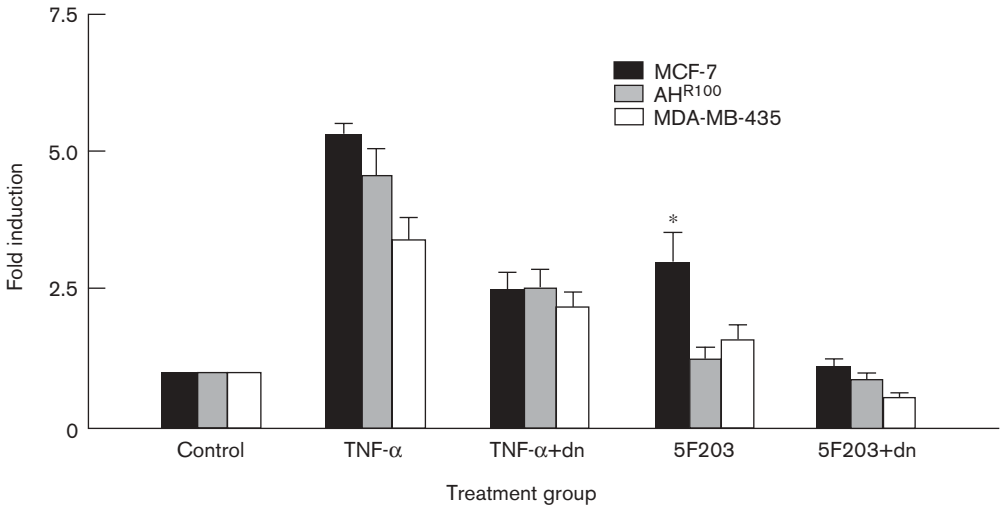
Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

Fig. 2



5F 203 increases NF-κB DNA binding activity in MCF-7 cells. EMSA was performed using an oligonucleotide which contains the consensus binding site for NF-κB. As described in Materials and methods, nuclear extracts from MCF-7 (A) and MDA-MB-435 (B) cells treated with 0.1% DMSO (negative control), TNF-α (positive control) or 5F 203 at varying time points were examined for NF-κB DNA binding activity. Numeric values listed at the bottom of blots represent fold induction. Data are representative of three separate experiments.

Fig. 3



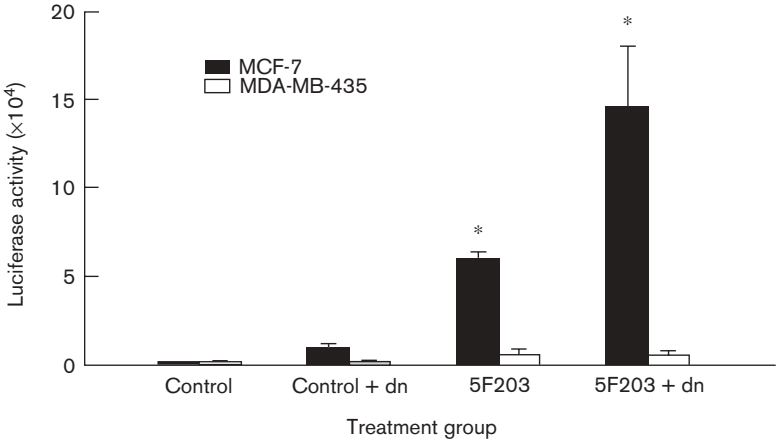
5F 203 induces NF-κB transcriptional activity in MCF-7 cells. MCF-7, AH^{R100} and MDA-MB-435 cells transfected with *R. reniformis* luciferase, NF-κB and NF-κB (empty vector) plasmid in the presence or absence of IκB dominant negative (dn) plasmid were treated with 0.1% DMSO (negative control, 8 h), 5F 203 (1 μM, 8 h) or TNF-α (positive control, 10 ng/ml, 3 h). Cells were harvested and luciferase activity was subsequently measured. Data represent mean ± SEM of at least three independent experiments. *$p < 0.05$, treated cells versus untreated cells.

5F 203 induces IL-6 gene expression in MCF-7 cells

MCF-7 cells were treated with 5F 203 and the mRNA level of the NF-κB-controlled gene IL-6 was determined by QRT-PCR. As shown in Fig. 5(A), there was a significant, dose-dependent increase in IL-6 mRNA expression in MCF-7 cells treated with 5F 203. We also

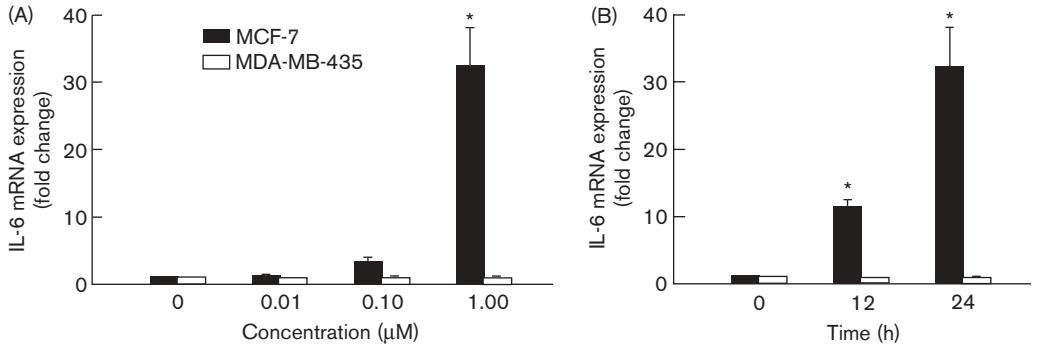
Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

Fig. 4



5F 203 treatment and inhibition of NFκB activation increases XRE-driven luciferase activity in MCF-7 cells. MCF-7 and MDA-MB-435 cells plated (2×10^5 cells/well) in six-well plates were transfected with Lipofectamine using *R. reniformis* and XRE plasmid or plasmid without XRE sequence (empty vector serving as negative control) in the presence or absence of IκB dominant negative (dn). Cells were then treated with 1 μM 5F 203 or 0.1% DMSO (control) for 9 h, harvested, and luciferase activity was subsequently measured as described in Materials and methods. Data represent mean ± SEM obtained from three independent experiments performed in triplicate. *$p < 0.05$, treated cells versus untreated cells.

Fig. 5



5F 203 induces IL-6 gene expression in sensitive MCF-7 cells. RNA was extracted from MCF-7 cells treated with 0.01, 0.1 or 1.0 μM 5F 203 for 24 h while control samples received 0.1% DMSO (A). Alternatively, RNA was extracted from MCF-7 cells treated with 1.0 μM 5F 203 for 12 or 24 h with the control sample represented as treatment at 0 h (B). Extracted RNA was then evaluated using QRT-PCR in accordance with Materials and methods. Data represent mean ± SEM obtained from three independent experiments. *$p < 0.05$, treated cells versus untreated cells.

detected a significant time-dependent increase in IL-6 mRNA expression (Fig. 5B). No appreciable increase in IL-6 mRNA occurred in MDA-MB-435 cells when treated with 5F 203. The mRNA levels of another NF-κB-dependent gene, IL-8, was also measured, but no increase was detected for either cell line following 5F 203 treatment (data not shown).

Discussion

In this study we show that 5F 203 (Fig. 1) demonstrates promising anticancer activity with its lysyl amide prodrug currently slated to begin clinical trials. The anticancer activity of 5F 203 depends on the ability of CYP1A1 to metabolically activate this drug candidate into DNA-damaging metabolites [19]. In addition, 5F 203 and/or its metabolites form DNA adducts in the form of single-strand breaks and DNA–protein cross-links (Brantley, manuscript in preparation) which enables the tumor cell to initiate apoptosis [4,19]. This DNA damage is selective, occurring only in certain cancer cell lines.

Recently, it has been demonstrated that 5F 203's ability to inhibit growth of cancer cells is correlated with its ability to induce CYP1A1 expression [1]. However, most cell lines have little or no constitutive CYP1A1 expression. As previously demonstrated, 5F 203 circumvents this problem by activating the AhR, which increases the transcription rate of the CYP1A1 gene, ultimately

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

increasing CYP1A1 enzyme levels [4]. Sensitivity of cancer cells to 5F 203 is therefore dependent on a functional, activated AhR. However, small molecules that activate AhR have also been shown to trigger NF-κB signaling [11].

Activation of the NF-κB signaling pathway results in the release of NF-κB from its inactive, cytosolic complex, into a nuclear, DNA-binding form. Using EMSA, we found that treatment of MCF-7 cells with 5F 203 induced a translocation of NF-κB from the cytosol to the nucleus, where it specifically binds to NF-κB consensus sequences. However, activation of NF-κB signaling did not occur in 5F 203-treated MDA-MB-435 cells. Since these cells do not have a functional AhR, this suggests that NF-κB activation by 5F 203 is dependent on the AhR.

NF-κB binding to its consensus sequence in target genes does not always lead to an increase in transcription [20,21]. We used a reporter vector containing the κB consensus sequence to determine whether 5F 203 increased NF-κB-dependent transcription. We showed that 5F 203 treatment of MCF-7 cells led to a significant increase in transcription in sensitive MCF-7 cells, but not in insensitive MDA-MB-435 or AH^{R100} cells. This is in agreement with the EMSA data. The specificity of this increase was demonstrated by using a dominant negative NF-κB, which ablated the 5F 203-induced increase in transcription in MCF-7 cells.

The expression of a number of genes is induced by NF-κB. We selected the cytokines IL-6 and IL-8 for this study. 5F 203 treatment of MCF-7 cells caused a dose- and time-dependent increase in IL-6 mRNA expression, demonstrating that the expression of NF-κB target genes is indeed stimulated by 5F 203 treatment. However, IL-8 mRNA expression was not increased by 5F 203, suggesting that NF-κB signaling caused by 5F 203 is selective.

Our data also suggests that there is a physical interaction of NF-κB with the AhR. Treatment of cells with 5F 203 stimulated NF-κB translocation and DNA binding. However, if the nuclear extracts were treated with an antibody to the AhR, binding was eliminated. Furthermore, transfection of cells with the dominant negative NF-κB caused a hyperresponse in XRE-mediated transcription in cells treated with 5F 203, suggesting that NF-κB acts as a negative regulator of AhR activity.

The present study clearly demonstrates that the anticancer drug candidate 5F 203 activates NF-κB signaling in sensitive MCF-7 cells that results in a selective increase in the expression of NF-κB target genes. However, NF-κB triggers a number of genes that would tend to promote cancer cell survival and activation of NF-κB, which may thus be considered detrimental. On the other hand, NF-κB activation is essential to a number of physiological processes (i.e. embryonal liver development, liver regeneration and DNA replication) that depend on this pathway [22,23]. In addition, treatment of breast cancer cells with Mullerian inhibiting substance activated the NF-κB pathway and subsequently suppressed the growth of these cells [8]. In this investigation, 5F 203 potently inhibited MCF-7 cancer cell growth and therefore any cell survival pathways that are upregulated by 5F 203 are apparently overriden by the DNA damage that it causes in sensitive cells. Furthermore, the role of NF-κB in cancer cell survival is unclear and it has recently been suggested that NF-κB may be pro-apoptotic [24]. It is plausible that 5F 203 induces apoptosis in sensitive cells by a mechanism that might involve NF-κB activation.

In conclusion, the present study demonstrates the ability of 5F 203 to activate NF-κB signaling in sensitive MCF-7 cells. 5F 203 may activate NF-κB in response to DNA damage in addition to directly mediating its antiproliferative activity. Additional studies are needed to provide further insight into the mechanism of anticancer action for 5F 203.

Acknowledgments

We thank Drs Willie Davis and Matthew Young for their valuable comments and suggestions.

References

1 Hose CD, Hollingshead M, Sausville EA, Monks A. Induction of CYP1A1 in tumor cells by the antitumor agent 2-[4-amino-3-methylphenyl]-5-fluorobenzothiazole: a potential surrogate marker for patient sensitivity. *Mol Cancer Ther* 2003; **2**:1265–1272.

2 Shi DF, Bradshaw TD, Wrigley S, McCall CJ, Lelieveld P, Fichtner I, *et al.* Antitumor benzothiazoles. 3. Synthesis of 2-(4-aminophenyl)benzothiazoles and evaluation of their activities against breast cancer cell lines *in vitro* and *in vivo*. *J Med Chem* 1996; **39**:3375–3384.

3 Hutchinson I, Chua MS, Browne HL, Trapani V, Bradshaw TD, Westwell AD, *et al.* Antitumor benzothiazoles. 14. Synthesis and *in vitro* biological properties of fluorinated 2-(4-aminophenyl)benzothiazoles. *J Med Chem* 2001; **44**:1446–1455.

4 Trapani V, Patel V, Leong CO, Ciolino HP, Yeh GC, Hose C, *et al.* DNA damage and cell cycle arrest induced by 2-(4-amino-3-methylphenyl)-5-fluorobenzothiazole (5F 203, NSC 703786) is attenuated in aryl hydrocarbon receptor deficient MCF-7 cells. *Br J Cancer* 2003; **88**: 599–605.

5 Leong CO, Gaskell M, Martin EA, Heydon RT, Farmer PB, Bibby MC, *et al.* Antitumour 2-(4-aminophenyl)benzothiazoles generate DNA adducts in sensitive tumour cells *in vitro* and *in vivo*. *Br J Cancer* 2003; **88**:470–477.

6 Rouet-Benzineb P, Aparicio T, Guilmeau S, Pouzet C, Descatoire V, Buyse M, *et al.* Leptin counteracts sodium butyrate-induced apoptosis in human colon cancer HT-29 cells via NF-kappaB signaling. *J Biol Chem* 2004; **279**:16495–16502, 2004.

7 Dawson MI, Hobbs PD, Peterson VJ, Leid M, Lange CW, Feng KC, *et al.* Apoptosis induction in cancer cells by a novel analogue of 6-[3-(1-adamantyl)-4-hydroxyphenyl]-2-naphthalenecarboxylic acid lacking retinoid receptor transcriptional activation activity. *Cancer Res* 2001; **61**: 4723–4730.

8 Segev DL, Ha TU, Tran TT, Kenneally M, Harkin P, Jung M, *et al.* Mullerian inhibiting substance inhibits breast cancer cell growth through an NFkappa B-mediated pathway. *J Biol Chem* 2000; **275**:28371–28379.

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

9 Bonnard M, Mirtsos C, Suzuki S, Graham K, Huang J, Ng M, *et al.* Deficiency of T2K leads to apoptotic liver degeneration and impaired NF-kappaB-dependent gene transcription. *EMBO J* 2000; **19**:4976–4985.
10 Cory AH, Cory JG. Lactacystin, a proteasome inhibitor, potentiates the apoptotic effect of parthenolide, an inhibitor of NFkappaB activation, on drug-resistant mouse leukemia L1210 cells. *Anticancer Res* 2002; **22**:3805–3809.
11 Jana NR, Sarkar S, Ishizuka M, Yonemoto J, Tohyama C, Sone H. Cross-talk between 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and testosterone signal transduction pathways in LNCaP prostate cancer cells. *Biochem Biophys Res Commun* 1999; **256**:462–468.
12 Kim DW, Gazourian L, Quadri SA, Romieu-Mourez R, Sherr DH, Sonenshein GE. The RelA NF-kappaB subunit and the aryl hydrocarbon receptor (AhR) cooperate to transactivate the c-*myc* promoter in mammary cells. *Oncogene* 2000; **19**:5498–5506.
13 Ciolino HP, Dankwah M, Yeh GC. Resistance of MCF-7 cells to dimethylbenz[*a*]anthracene-induced apoptosis is due to reduced CYP1A1 expression. *Int J Oncol* 2002; **2**:385–391.
14 Hoivik D, Willett K, Wilson C, Safe S. Estrogen does not inhibit 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-mediated effects in MCF-7 and Hepa 1c1c7 cells. *J Biol Chem* 1997; **272**:30270–30274.
15 Chua MS, Kashiyama E, Bradshaw TD, Stinson SF, Brantley E, Sausville EA, *et al.* Role of Cyp1A1 in modulation of antitumor properties of the novel agent 2-(4-amino-3-methylphenyl)benzothiazole (DF 203, NSC 674495) in human breast cancer cells. *Cancer Res* 2000; **60**: 196–5203.
16 Brown K, Franzoso G, Baldi L, Carlson L, Mills L, Lin Y-C, *et al.* The signal response of IkBalpha is regulated by transferable N- and C-terminal domains. *Mol Cell Biol* 1997; **17**:3021–3027.
17 Loaiza-Perez AI, Trapani V, Hose C, Singh SS, Trepel JB, Stevens MFG, *et al.* Aryl hydrocarbon receptor mediates sensitivity of MCF-7 breast cancer cells to antitumor agent 2-(4-amino-3-methylphenyl) benzothiazole. *Mol Pharmacol* 2002; **61**:13–19.
18 Andrews NC, Faller DV. A rapid micropreparation technique for extraction of DNA-binding proteins from limiting numbers of mammalian cells. *Nucleic Acids Res* 1991; **19**:2499.
19 O'Brien SE, Browne HL, Bradshaw TD, Westwell AD, Stevens MF, Laughton CA. Antitumor benzothiazoles. Frontier molecular orbital analysis predicts bioactivation of 2-(4-aminophenyl)benzothiazoles to reactive intermediates by cytochrome P4501A1. *Org Biomol Chem* 2003; **1**: 493–497.
20 Camarda G, Binaschi M, Maggi CA, Goso C. Nuclear factor-kappaB. induced in human carcinoma cell line A2780 by the new anthracycline men 10755, is devoid of transcriptional activity. *Int J Cancer* 2002; **102**: 476–482.
21 Smartt HJ, Elder DJ, Hicks DJ, Williams NA, Paraskeva C. Increased NF-kappaB DNA binding but not transcriptional activity during apoptosis induced by the COX-2-selective inhibitor NS-398 in colorectal carcinoma cells. *Br J Cancer* 2003; **89**:1358–1365.
22 Kuhnel F, Zender L, Paul Y, Tietze MK, Trautwein C, Manns M, *et al.* NFkappaB mediates apoptosis through transcriptional activation of Fas (CD95) in adenoviral hepatitis. *J Biol Chem* 2000; **275**:6421–6427.
23 Kirillova I, Chaisson M, Fausto N. Tumor necrosis factor induces DNA replication in hepatic cells through nuclear factor kappaB activation. *Cell Growth Differ* 1999; **10**:819–828.
24 Sonis ST. The biologic role for nuclear factor-kappaB in disease and its potential involvement in mucosal injury associated with anti-neoplastic therapy. *Crit Rev Oral Biol Med* 2002; **13**:380–389.

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.