

Award Number: W81XWH-07-1-0081

TITLE: Curcumin Based Drug Screening for Inhibitors of NF kappa B in a Cell Model of Prostate Cancer Progression

PRINCIPAL INVESTIGATOR: Marco Bisoffi

CONTRACTING ORGANIZATION: University of New Mexico School of Medicine
Albuquerque, NM 87131

REPORT DATE: February 2008

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGEForm Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**

1. REPORT DATE (DD-MM-YYYY) 01-02-2008		2. REPORT TYPE Final		3. DATES COVERED (From - To) 1 FEB 2007 - 31 JAN 2008	
4. TITLE AND SUBTITLE Curcumin Based Drug Screening for Inhibitors of NF kappa B in a Cell Model of Prostate Cancer Progression				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-07-1-0081	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Marco Bisoffi E-Mail: mbisoffi@salud.unm.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of New Mexico School of Medicine Albuquerque, NM 87131				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT We have identified structurally diverse chemical analogs of the polyphenolic phytochemical Curcumin with an enhanced inhibitory effect against hallmarks of prostate cancer progression. In the androgen independent and bone metastatic cell model of prostate cancer C4-2, these analogs inhibited (i) the activation of the nuclear transcription factor NF kappa B; (ii) cell proliferation, (iii) anchorage independent growth, (iv) the expression of Interleukin-6, and (v) the activation of the androgen receptor. In particular, polyphenols with 5-carbon linkers (analog #48 and #50) and nitrogen side groups (analog #50) were more efficient than the mother compound Curcumin in inhibiting several hallmarks of prostate cancer progression. The analogs covered in this study display a variety of structural changes, including the length of the carbon chain, the nature of the side groups and the number of the aryl rings. This variety represents a platform for more refined screens of subtle variations of the analogs reported here; these screens will be performed in assays specific for the measurement of several molecular markers representing prostate cancer progression and bone metastasis, including the ones reported here and others, such as migration, invasion, adhesion to bone endothelial cells, and mineralization.					
15. SUBJECT TERMS Prostate cancer progression, Curcumin analogs, Interleukin-6, androgen receptor, NF kappa B, cell proliferation, anchorage independent growth					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U			USAMRMC
			UU	12	19b. TELEPHONE NUMBER (include area code)

Table of Contents

	<u>Page</u>
Introduction.....	4
Body.....	4
Key Research Accomplishments.....	9
Reportable Outcomes.....	9
Conclusion.....	9
References.....	11
Appendices.....	12

Curcumin Based Drug Screening for Inhibitors of NFκB in a Cell Model of Prostate Cancer Progression
 Final Progress Report for DOD - PC060864

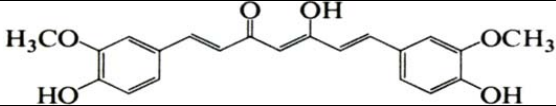
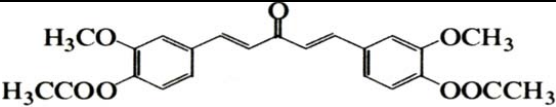
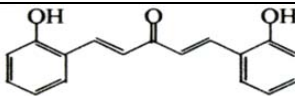
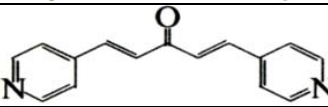
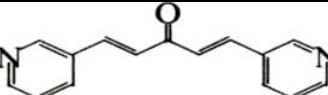
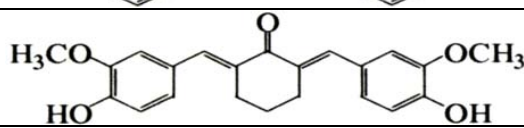
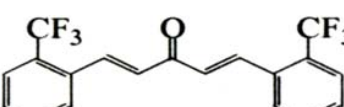
INTRODUCTION

The overall and continuous goal of our research funded by the DOD PC060864 Concept Award is to identify new and structurally diverse chemical analogs of the polyphenolic phytochemical Curcumin from the Indian herb *Curcuma longa* (family *Zingiberaceae*) active against advanced prostate cancer. For this, we are using cells and experimental conditions that mimic low or absent androgen concentrations and the bone microenvironment, as often encountered in a patient undergoing androgen ablation and suffering from metastatic disease. Our assays cover both molecular as well as functional markers, of progression, including the expression and activation of proteins involved in autocrine stimulation, as well as phenotypic behavior relating to migration, invasion, and mineralization. Furthermore, it is our goal to use cheminformatic approaches to identify structurally defined analogs of Curcumin with an improved therapeutic efficacy against prostate cancer cells of the advanced phenotype. Finally, such analogs will then be subjected to pre-clinical toxicity studies in animals, and further considered for clinical development.

BODY

The research team of DOD PC060864 has access to a chemical library of Curcumin analogs consisting of >100 structurally characterized compounds. For part of this library, collaborators Drs. David Vander Jagt, Lorraine Deck, and Robert Orlando have generated structural activity relationship (SAR) data pertaining to NFκB activity, perhaps the most prominent target pathway of Curcumin [1,2]. This SAR data was generated using a HEK-293T cell line transfected with an NFκB promoter mediated Luciferase reporter construct available from Panomics (Redwood City, CA). In this system, NFκB was induced by tumor necrosis factor α (TNFα) at a concentration of 20ng/ml. Table 1 lists the inhibitory concentration for Curcumin and each of the compounds tested at which 50% of the NFκB activity was inhibited, i.e, the IC₅₀ values.

Table 1: NFκB IC₅₀ values (triplicate ± SD) for Curcumin and six chemical analogs using the HEK-293T Luciferase reporter system.

Compound	Structure	IC ₅₀ in μM
Curcumin		8.2 ± 0.4
Analog #46		5.3 ± 1.1
Analog #48		4.2 ± 0.3
Analog #49		3.9 ± 0.3
Analog #50		3.4 ± 0.2
Analog #54		4.4 ± 0.8
Analog #67		5.0 ± 0.3

The NF kappa B specific SAR data served as a rationale for engaging the corresponding Curcumin analogs in several lines of research that are in agreement with the specific aims of PC060864. This report covers the following aspects: (i) Cell growth inhibition; (ii) anchorage independent colony formation; (iii) androgen receptor (AR) activation; (iv) Interleukin-6 (IL-6) expression, and Akt/PKB expression and activation. Our major tools in these investigations continue to be prostate epithelial cells from the LNCaP progression model, i.e. LNCaP, C4-2, and C4-2B [3,4]. As a brief reminder, LNCaP is androgen dependent for growth and survival and does not exert metastatic potential, while the derivative sublines C4-2 and C4-2B have developed androgen independence and (bone) metastatic potential (see Figure 1 in PC060864).

Cell Growth / Proliferation

LNCaP, C4-2, and C4-2B were subjected to the metabolic WST-1 formazan salt assay (Roche Pharmaceuticals, Nutley, NJ) growth assay in the presence of increasing concentrations of Curcumin and analogs at a 0-200 μM range. The IC_{50} values were determined for 24 hrs by non-linear regression and are displayed in Table 2. Analog #67 was excluded from these studies because of its strong insolubility in aqueous solutions at concentrations $>50 \mu\text{M}$ (Figure 1).

Table 2: Growth IC_{50} values (triplicate \pm SD) for Curcumin and five chemical analogs using the WST-1 formazan salt assay.

	LNCaP	C4-2	C4-2B
Curcumin	66 \pm 0.1	96 \pm 0.3	79 \pm 0.1
Analog #46	65 \pm 0.2	60 \pm 0.2	57 \pm 0.1
Analog #48	72 \pm 0.2	112 \pm 0.2	90 \pm 0.1
Analog #49	47 \pm 0.1	53 \pm 0.1	65 \pm 0.2
Analog #50	53 \pm 0.1	48 \pm 0.2	69 \pm 0.2
Analog #54	60 \pm 0.1	71 \pm 0.1	108 \pm 0.1

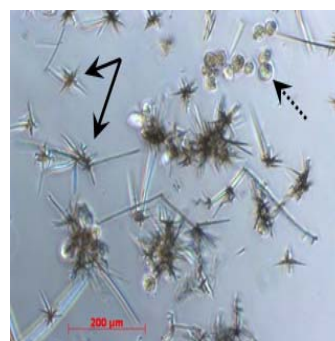
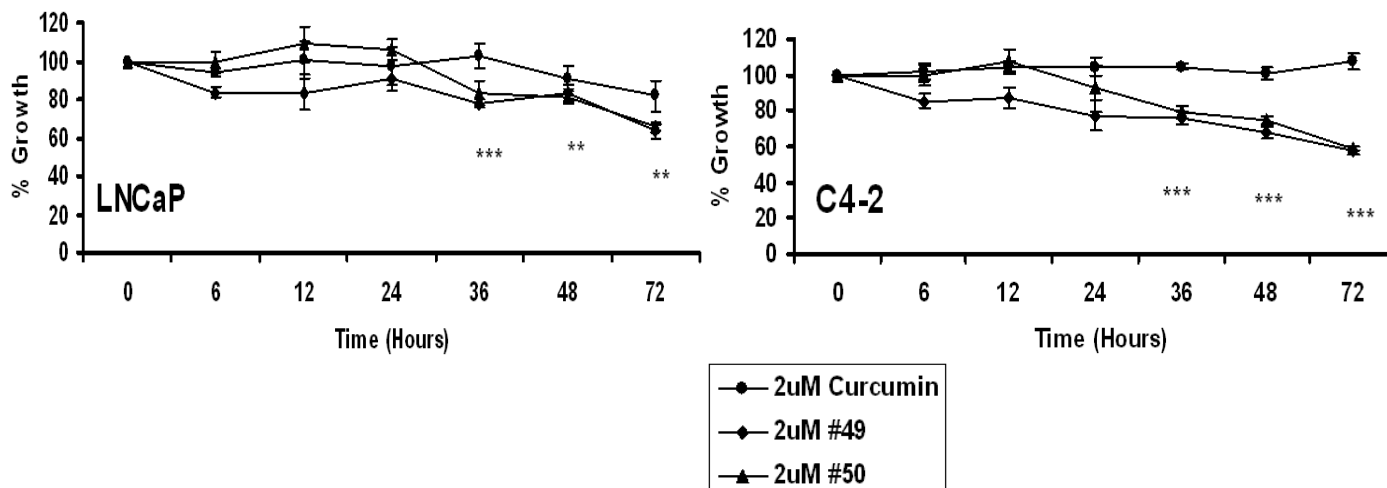


Figure 1: Crystal formation of Curcumin Analog #67 in aqueous media at $>50 \mu\text{M}$ concentration; solid arrows point at crystals; dotted arrow points at LNCaP cells.

The concentrations listed in Table 2 are far from being physiologically reachable. In fact, even though Curcumin's excellent safety and tolerability has been shown in Phase I clinical trials with up to 12 grams (!!) of daily doses [5-8], Curcumin's greatest pitfall that has hindered its transition to large scale Phase III clinical trials is its low bioavailability, with typically 2-3% of the given dose reaching the blood and tissues. This is mainly due to Curcumin's hydrophobic nature (see for example analog #67 in Figure 1), and to its rapid metabolism to glucuronide and sulphate derivatives, and ferulic and dihydroferulic acid [9]. Nevertheless, we tested Curcumin and two of its structural analogs at the physiological concentration of 2 μM over a period of 72 hrs. Analogs #49 and #50 were chosen due to their lower IC_{50} values in C4-2 compared to LNCaP (see Table 2). The data in Figure 2 shows that starting from 36 hrs the analogs show an increased inhibition as compared to Curcumin in all the prostate cancer cell lines tested.



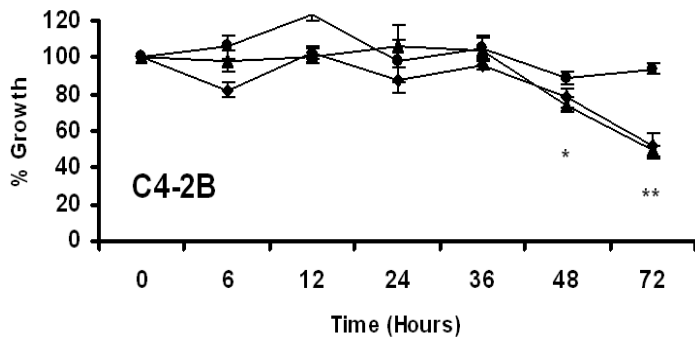


Figure 2: Relative growth rates (% of untreated control, i.e. 0.1% DMSO) of LNCaP, C4-2, and C4-2B treated with 2 μM Curcumin, and Analogs #49 and #50. Data represent averages of 6 experiments ± SD; *, **, *** indicate difference from Curcumin at $p \leq 0.05$, 0.01, 0.001, respectively (t-test).

Anchorage Independent Colony Formation

Anchorage independent colony formation represents a hallmark of aggressive growth and proliferation as displayed by C4-2B cells. Using colony formation in soft agar covered by growth medium, we exposed C4-2 cells to 40 μM Curcumin and the five structural analogs listed in Table 2. Effects on cell growth were visible at 5-7 days, and the experiments were finalized at 21 days. Figure 3A shows a qualitative assessment of the inhibitory effect as shown by conventional light microscopy. The data was also quantified by counting the number of colonies in three randomly chosen microscopic fields, which allows calculating the inhibitory effect in % of untreated control (0.1% DMSO) as show in Figure 3B. In this analysis, all of the treatment groups were significantly different from the control ($p < 0.05$). In addition, analogs #48 and #50 were also significantly more inhibitory than Curcumin ($p < 0.05$).

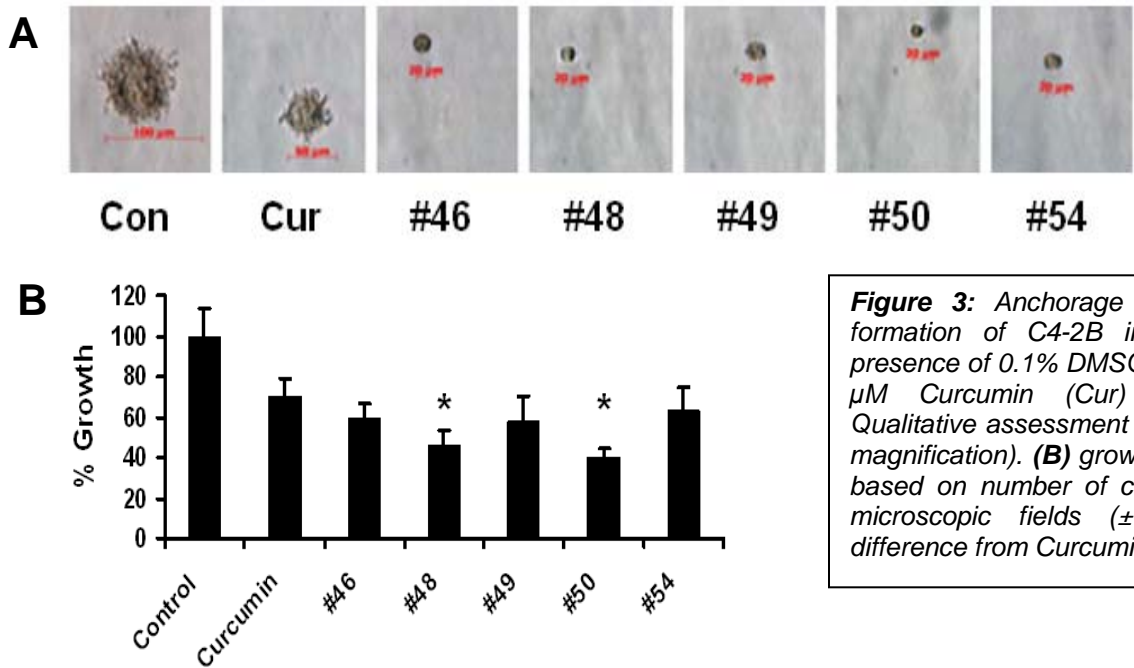
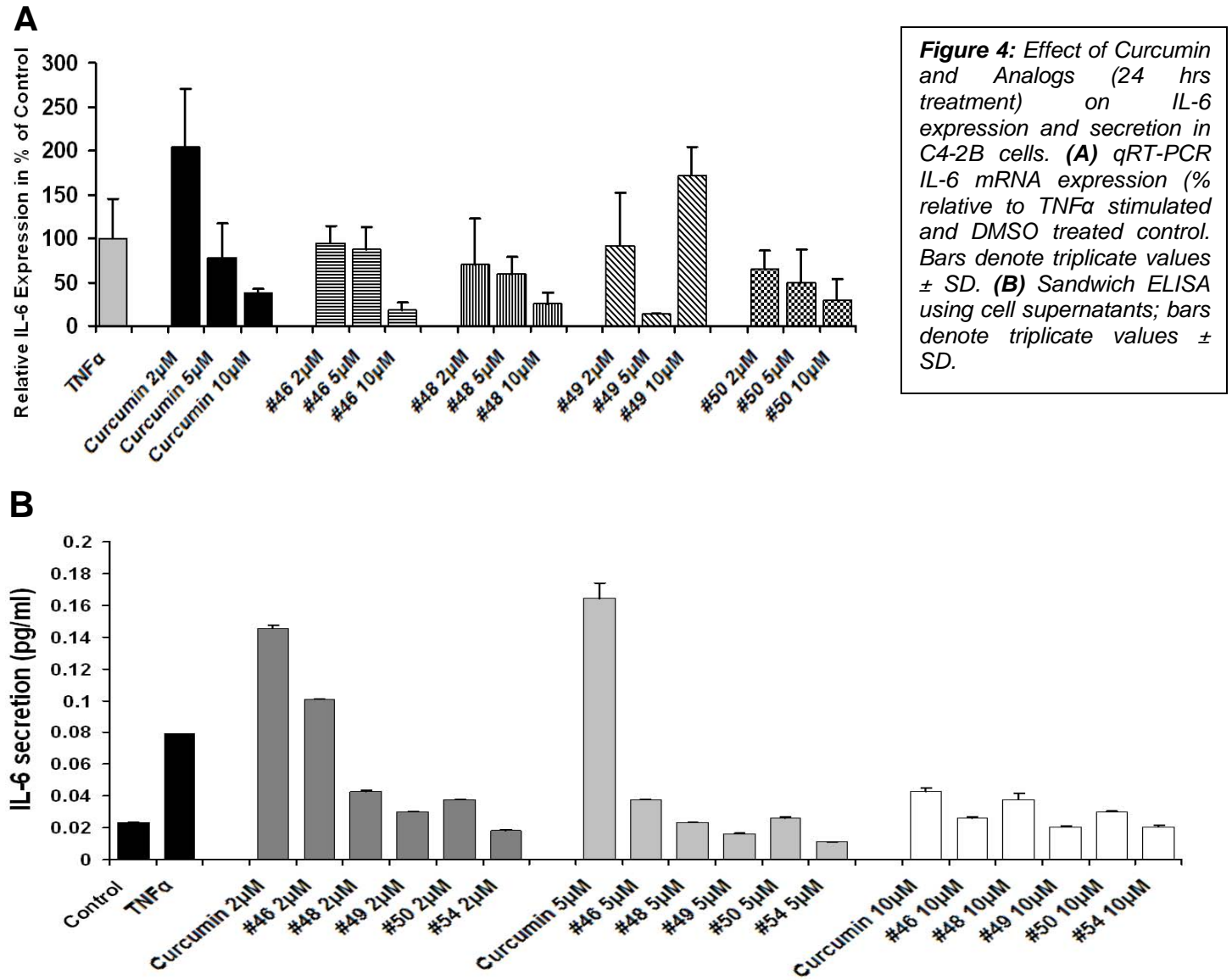


Figure 3: Anchorage independent colony formation of C4-2B in soft agar in the presence of 0.1% DMSO (control, Con) or 40 μM Curcumin (Cur) or Analogs. (A) Qualitative assessment by microscopy (x250 magnification). (B) growth rate (% of control) based on number of colonies in 3 random microscopic fields (± SD). * indicates difference from Curcumin at $p < 0.05$ (t-test).

Interleukin-6 Expression

During this funding period, we have developed a strong interest in the cytokine Interleukin-6 (IL-6). IL-6 plays a central role in host defense due to its wide range of immune activities. Pertinent to the present investigation, IL-6 has been shown to be elevated in the serum of prostate cancer patients [10-13]. In addition, expression of IL-6 in prostate cancer cells represents an autocrine stimulatory loop that leads to non-steroidal activation of the androgen receptor (AR) [14,15]. In addition, IL-6 is a downstream target of the transcription factor NFκB [16]. We have thus studied the effect of Curcumin and its analogs on the expression of IL-6 by two different methods, i.e. quantitative (real-time) reverse transcriptase polymerase chain reaction (qRT-PCR), and by enzyme linked immunosorbent assay (ELISA).

The data in Figure 4A represents IL-6 mRNA expression in C4-2B cells treated for 24 hrs with Curcumin and its analogs relative to untreated control stimulated with the NFκB activator tumor necrosis factor alpha (TNFα). In general, a dose dependent response was observed between a range of 2 and 10 μM of added drugs, although substantial variation was evident, such as in the case of analog #49. Curcumin seems to be stimulatory for IL-6 at low concentrations, an observation also often made for other molecular outcomes with other polyphenolic phytochemicals, such as Resveratrol (our own experience, not shown here). IL-6 is a cytokine acting in a paracrine or autocrine manner; for its biological activity to be fully exerted it must thus be secreted. The effect of Curcumin and its analogs at the same concentrations were tested for their effects on IL-6 secretion using supernatants from cells treated for 24 hrs. For increased sensitivity, a sandwich ELISA was used (eBioscience, San Diego, CA). Interestingly, all analogs were more efficient in inhibiting IL-6 secretion than Curcumin at concentrations of 2 and 5 μM, while at 10 μM Curcumin was as effective. Of note, and in agreement with the qRT-PCR data, Curcumin at low concentrations was again somewhat stimulatory (Figure 4B).



Akt Expression and Activation

It has been proposed by the Kreisberg laboratory that progression of prostate cancer to androgen independence is accompanied by a switch in signal transduction pathways mediating cell growth and survival [17,18]. The classical mitogen activated protein kinase (MAPK) pathway resulting in the activation of the transcription factor ERK1/2 (extracellular signal regulated kinase) is prominent in LNCaP cells, while in C4-2

the Akt/PKB (protein kinase B) and mTOR (mammalian target of Rapamycin) pathways gain importance for the growth and survival of the cells [18]. Consequently, LNCaP cells are insensitive to the actions of the bacterial macrolide Rapamycin, while the growth and survival of C4-2 cells are greatly inhibited. It has been previously shown that Curcumin inhibits Akt expression and phosphorylation (activation), albeit at rather high concentrations, i.e. 20-30 μM [19]. In agreement with these reports, our own data generated by Western blot analysis of C4-2 cells treated with Curcumin showed a constitutively active Akt with little or no change after stimulation with the mitogen insulin-like growth factor 1 (IGF-1)(Figure 5A). Phosphorylation of Akt is dependent on upstream activation of phosphatidylinositol 3-phosphate kinase (PI3K); inhibition of PI3K with subsequent inhibition of Akt activation was shown by applying the specific small molecule PI3K inhibitor LY294002. Curcumin started to inhibit Akt phosphorylation at 20 μM . Part of this inhibition is due to change in expression levels of Akt, which was affected by Curcumin during the total incubation time of 6 hrs. To test the effect of Curcumin and its chemical analogs on Akt phosphorylation specifically, the cells were treated for 20 min only at a concentration of 20 μM (Figure 5B). Under these conditions, total Akt was largely unaffected. In addition, some of the analogs, such as for example #48, were more inhibitory for Akt phosphorylation than Curcumin itself.

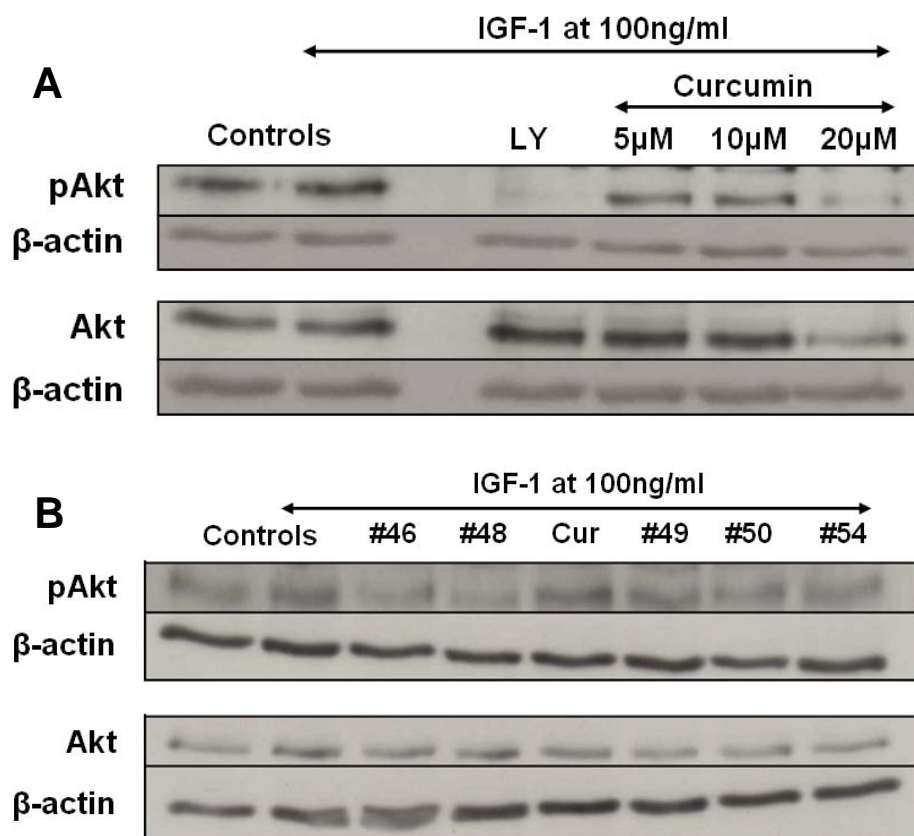


Figure 5: Effect of Curcumin and analogs on Akt expression and activation by Western blot analysis. **(A)** Cells were treated with Curcumin for 4 hrs, then stimulated with IGF-1 at 100 ng/ml for 1 hr; the PI3K inhibitor LY294002 (LY) was used at 20 μM for 1 hr. **(B)** Cells were treated with Curcumin and analogs at 20 μM and IGF-1 at 100 ng/ml simultaneously for 20 min.

Androgen Receptor Trans-Activation

Activation of the AR in most prostate tumors is an indispensable requirement for cell survival and growth; thus the AR is a prominent therapeutic target [20,21]. The latter is also true for progressive disease; for example, while androgen independence indicates that androgens are no longer necessary, activation of the AR still is [18,21]. We are thus interested in identifying Curcumin analogs that are capable of inhibiting AR expression. As shown in Figure 6, we have so far generated data pertaining to Curcumin. The effect of Curcumin on AR was analyzed in the AR-negative prostate cancer cell line PC-3 double-transfected with a plasmid expressing the wild type human AR (hAR) under the control of a CMV promoter, and an MMTV promoter driven Enhanced Green Fluorescence Protein (EGFP) construct; the MMTV sequence contains an androgen responsive element (ARE) which is activated by androgen leading to expression of EGFP. EGFP expression can be visualized by fluorescence microscopy (Figure 6A) and quantified using ImageJ software (<http://rsb.info.nih.gov/ij/>)(Figure 6B). CMV-hAR and MMTV-EGFP transfected PC-3 cells were starved for 24

hrs and treated with the synthetic androgen R1881 at a 0.5 pM concentration in the absence (control) or presence of increasing concentrations of Curcumin (0.5 – 15 μ M) for 24 hrs. In these experiments, Curcumin inhibited AR trans-activation in a dose responsive manner (Figure 6B). We are currently repeating these experiments using LNCaP and C4-2 cells and engaging the Curcumin analogs listed in Table 1.

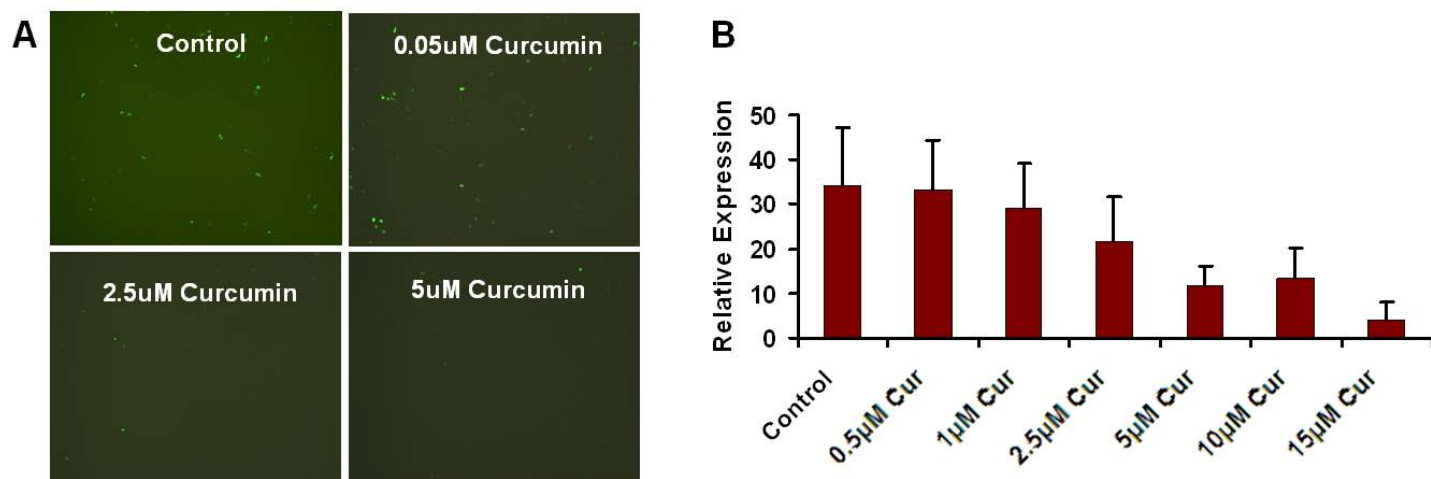


Figure 6: Effect of Curcumin on AR trans-activation. **(A)** Fluorescence microscopy of PC-3 cells transfected with CMV-hAR and MMTV-EGFP and stimulated with 0.5 pM synthetic androgen R1881 in the absence (control) or presence of increasing concentrations of Curcumin. **(B)** Quantification of EGFP expression using ImageJ software (<http://rsb.info.nih.gov/ij/>); bars indicate fluorescence intensity in three randomly chosen microscopic fields \pm SD.

KEY RESEARCH ACCOMPLISHMENTS

- Characterization of the effect of Curcumin on molecular and cellular hallmarks of prostate cancer progression.
- Identification of novel chemical analogs of Curcumin with an effect on markers of prostate cancer progression.

REPORTABLE OUTCOMES

- Training of Graduate Student (Ms. Alexandra Fajardo), a Pfizer Safety Fellowship Awardee on the use of polyphenolic phytochemicals in *in vitro* culture of prostate cancer cells.
- Manuscript in preparation: “Chemical Analogs of Curcumin Effective against Prostate Cancer Progression”, to be submitted to “The Prostate”, “Journal of Urology”, etc.
- Poster Presentation (Fajardo et al) at the 25th Annual Meeting of the Mountain West Society of Toxicology in Breckenridge, CO in September 2007: “Identification of Curcumin Analogs Toxic against Prostate Cancer Cells Through Inhibition of the Pro-Survival Nuclear Factor Kappa B Transcription Factor”.
- Poster Presentation (Fajardo et al; as above) at the Next Generation Sequencing Symposium, Santa Fe, NM in March 2008, organized by New Mexico INBRE, New Mexico Tech, Sandia National Laboratories, and Los Alamos National Laboratories.
- NIH/NCI R21 grant application (1RO3CA136030-01), “Targeting Egr-1 with Curcumin Analogs for Prostate Cancer Prevention”; Bisoffi M. – PI.
- NIH/NCI R21 grant application (1R21CA-pending assignment), “Magnetic Targeting of Multifunctionalized SPIONs to Prostate Tumors”; Bisoffi M. – PI.

CONCLUSIONS

In summary, we have successfully initiated the testing of structurally diverse Curcumin analogs on hallmarks of prostate cancer progression, including cell growth inhibition, anchorage independent colony formation, androgen receptor (AR) trans-activation, Interleukin-6 (IL-6) expression, and Akt/PKB expression and

activation. In particular, polyphenols with 5-carbon linkers (analogs #48 and #50) and nitrogen side groups (analog #50) were more efficient than the mother compound Curcumin in inhibiting several hallmarks of prostate cancer progression. Our overall goal is to identify chemical analogs of this promising phytochemical that are inhibitory for hallmarks of cancer progression. Consequently, we have focused on the androgen independent and metastatic cell line C4-2B and have performed most of the reported experiments in the absence of androgen to mimic the conditions in a patient undergoing androgen ablation as part of his therapy for progressive disease. DOD PC060864 also proposed to add components of the bone microenvironment to mimic conditions of bone metastatic disease. Consequently, we will repeat these experiments either in the presence of cultured human fetal osteoblasts (hFOB) or conditioned media from these cells. In this context, we have successfully established hFOB cell cultures and are currently testing the analogs for some of the above listed markers under bone metastatic conditions.

Due to other research developments in our laboratories, we have focused here on the cytokine IL-6, which was not included in the original DOD PC060864 Concept Award application. However, IL-6 is reported to play a similar role as IL-8, which is part of the original application. In addition, we have initiated expression studies of IL-8, Osteopontin (OPN), and matrix metalloproteinases 2 and 9 (MMP-2 and MMP-9). However, we have so far not been able to establish experimentally reproducible assays for these markers; corresponding efforts are currently under way and our successful results for IL-6 serve as a guide for these experiments.

Finally, it should be noted that the analogs covered in this study display a variety of structural changes, including the length of the carbon chain, the nature of the side groups and the number of the aryl rings. This variety represents a platform for more refined screens of subtle variations of the analogs reported here; these screens can be performed in assays specific for the measurement of several molecular markers. In addition to these extended screens, we now plan to move towards Specific Aim 2 of DOD PC060864, i.e. to apply a cheminformatics approach encompassing quantitative structure-activity relationship (QSAR) and ligand-based virtual screening (LBVS) to explore the possibility of improving their efficacy. With this goal in mind, and upon further testing of additional compounds, as well as testing compounds under bone metastatic conditions, we will consult with the staff of the Biocomputing Division of the Department of Biochemistry and Molecular Biology led by Dr. Tudor Oprea.

The findings presented in this final report, together with data currently being generated from additional experiments under way in our laboratories are part of a planned manuscript; we envisage positive peer reviews from Journal such as "The Prostate" and "Journal of Urology". Ms. Alexandra Fajardo, a Graduate Student in my laboratory has presented part of this data at the 25th Annual Meeting of the Mountain West Society of Toxicology in Breckenridge, CO in September 2007. We will also use these data towards designing additional grant applications to be submitted to the NIH or DOD. In addition, it should be noted that the NCI-designated New Mexico Cancer Research and Treatment Center provides excellent support, through administrative, legal, and scientific mechanisms, to translate our findings towards clinical use. In particular, the Clinical and Translational Science Center (CTSC) is a guiding body for this endeavor.

Furthermore, the research funded by DOD PC060864 has fostered novel research avenues. For example, the insolubility problem observed for analog #67 shown in Figure 1 is part the low bioavailability of polyphenolic phytochemicals in pre-clinical animal and Phase I human clinical studies. We have initiated studies into the use of surface modified iron oxide superparamagnetic nanoparticles (SPIONs) functionalized to bind polyphenols and targeting antibodies (against prostate specific membrane antigen, PSMA) for better delivery to prostate tumors and for enhanced therapeutic effect through magnet guided tumor detainment. A corresponding NIH R21 grant application entitled "*Magnetic Targeting of Multifunctionalized SPIONs to Prostate Tumors*" has been recently submitted. A second application entitled "*Targeting Egr-1 with Curcumin Analogs for Prostate Cancer Prevention*" was submitted to the NIH/NCI and represents a combination based on the findings from DOD PC060864 and other research avenues in our laboratories.

The following personnel were/are involved in this research:

- Marco Bisoffi, PhD - Project management, data analysis
- Rob Orlando, PhD - NFkB activity measurements
- Tudor Oprea, MD/PhD - Cheminformatics

- Ming Ji - Experimental responsibilities
- Alexandra Fajardo - Experimental responsibilities

REFERENCES

1. Surh YJ, Chun KS. Cancer chemopreventive effects of curcumin. *Adv Exp Med Biol.* 2007; 595: 149-72.
2. Aggarwal BB, Sundaram C, Malani N, Ichikawa H. Curcumin: the Indian solid gold. *Adv. Exp. Med. Biol.* 2007; 595, 1–75.
3. Thalmann GN, Anezinis PE, Chang SM, Zhou HE, Kim EE, Hopwood VL, Pathak S, von Eschenbach AC, Chung LW. Androgen-independent cancer progression and bone metastasis in the LNCaP model of human prostate cancer. *Cancer Res* 1994; 54: 2577-81.
4. Thalmann GN, Sikes RA, Wu TT, Degeorges A, Chang SM, Ozen M, Pathak S, Chung LW. LNCaP progression model of human prostate cancer: androgen-independence and osseous metastasis. *Prostate* 2000; 44: 91-103.
5. Lao CD, Ruffin MT, Normolle D, Heath DD, Murray SI, Bailey JM, Boggs ME, Crowell J, Rock CL, Brenner DE. Dose escalation of a curcuminoid formulation. *BMC Complement Altern. Med.* 2006; 6, 10.
6. Cheng AL., Hsu CH, Lin JK, Hsu MM, Ho YF, Shen TS, Ko JY, Lin JT, Lin BR, Ming-Shiang W, Yu HS, Jee SH, Chen GS, Chen TM, Chen CA, Lai MK, Pu YS, Pan MH, Wang YJ, Tsai CC, Hsieh CY. Phase I clinical trial of curcumin, a chemopreventive agent, in patients with high-risk or pre-malignant lesions. *Anticancer Res.* 2001; 21 (4B), 2895–900.
7. Shoba G, Joy D, Joseph T, Majeed M, Rajendran R, Srinivas PS. Influence of piperine on the pharmacokinetics of curcumin in animals and human volunteers. *Planta Med* 1998; 64, 353–6.
8. Hsu CH, Cheng AL. Clinical studies with curcumin. *Adv. Exp. Med. Biol.* 2007; 595, 471–80.
9. Anand P, Kunnumakkara AB, Newman RA, Aggarwal BB. Bioavailability of Curcumin: Problems and Promises. *Mol Pharmacol.* 2007; 4, 807-816.
10. Shariat SF, Kattan MW, Traxel E, Andrews B, Zhu K, Wheeler TM, Slawin KM. Association of pre- and postoperative plasma levels of transforming growth factor beta(1) and interleukin 6 and its soluble receptor with prostate cancer progression. *Clin Cancer Res.* 2004; 10: 1992-9.
11. Shariat SF, Andrews B, Kattan MW, Kim J, Wheeler TM, Slawin KM. Plasma levels of interleukin-6 and its soluble receptor are associated with prostate cancer progression and metastasis. *Urology.* 2001; 58: 1008-15
12. Michalaki V, Syrigos K, Charles P, Waxman J. Serum levels of IL-6 and TNF-alpha correlate with clinicopathological features and patient survival in patients with prostate cancer. *Br J Cancer.* 2004; 90: 2312-6.
13. Nakashima J, Tachibana M, Horiguchi Y, Oya M, Ohigashi T, Asakura H, Murai M. Serum interleukin 6 as a prognostic factor in patients with prostate cancer. *Clin Cancer Res.* 2000; 6: 2702-6.
14. Culig Z, Bartsch G. Androgen axis in prostate cancer. *J Cell Biochem.* 2006; 99: 373-81.
15. Culig Z, Steiner H, Bartsch G, Hobisch A. Interleukin-6 regulation of prostate cancer cell growth. *J Cell Biochem.* 2005; 95: 497-505.
16. Paule B, Terry S, Kheuang L, Soyeux P, Vacherot F, de la Taille A. The NF-kappaB/IL-6 pathway in metastatic androgen-independent prostate cancer: new therapeutic approaches? *World J Urol.* 2007; 25: 477-89.
17. Immunohistochemical demonstration of phospho-Akt in high Gleason grade prostate cancer. Malik SN, Brattain M, Ghosh PM, Troyer DA, Prihoda T, Bedolla R, Kreisberg JI. *Clin Cancer Res.* 2002; 8: 1168-71.
18. Ghosh PM, Malik SN, Bedolla RG, Wang Y, Mikhailova M, Prihoda TJ, Troyer DA, Kreisberg JI. Signal transduction pathways in androgen-dependent and -independent prostate cancer cell proliferation. *Endocr Relat Cancer.* 2005; 12: 119-34.
19. Li M, Zhang Z, Hill DL, Wang H, Zhang R. Curcumin, a dietary component, has anticancer, chemosensitization, and radiosensitization effects by down-regulating the MDM2 oncogene through the PI3K/mTOR/ETS2 pathway. *Cancer Res.* 2007; 67: 1988-96.
20. Burnstein KL. Regulation of androgen receptor levels: implications for prostate cancer progression and therapy. *J Cell Biochem.* 2005; 95: 657-69.
21. Heinlein CA, Chang C. Androgen receptor in prostate cancer. *Endocr Rev.* 2004; 25: 276-308.

APPENDICES

Poster Presentations (Fajardo et al) at the:

- (i) 25th Annual Meeting of the Mountain West Society of Toxicology in Breckenridge, CO in September 2007.
- (ii) Next Generation Sequencing Symposium, Santa Fe, NM in March 2008, organized by New Mexico INBRE, New Mexico Tech, Sandia National Laboratories, and Los Alamos National Laboratories.

IDENTIFICATION OF CURCUMIN ANALOGS TOXIC AGAINST PROSTATE CANCER CELLS THROUGH INHIBITION OF THE PRO-SURVIVAL NUCLEAR FACTOR KAPPA B TRANSCRIPTION FACTOR

^{1,2}Alexandra Fajardo, ²Lucy Hunsacker, ³Lorraine Deck, ²David Vander Jagt, ¹Todd Thompson and ²Marco Bisoffi

¹ College of Pharmacy, Division of Toxicology, ² Department of Biochemistry and Molecular Biology, ³ Department of Chemistry, University of New Mexico

Prostate cancer is the most common malignancy to develop among men in the United States. The identification of agents that are selectively toxic against prostate cancer cells may be useful in prostate cancer treatment and prevention of prostate carcinogenesis. Curcumin, [1*E*,6*E*]-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione], is a phenolic phytochemical found in the South Asian spice turmeric which has previously been shown to have anti-inflammatory, anti-oxidant, anticoagulant and anti-carcinogenic properties. However, the *in vivo* bioavailability of curcumin is low, compromising its effectiveness as a cancer therapeutic agent. Consequently, novel synthetic curcumin analogs are under investigation as either chemopreventive or chemotherapeutic agents for a variety of cancers. In collaboration with investigators from the UNM Department of Chemistry, chemical libraries of curcumin analogs were synthesized and screened for their inhibitory action on Nuclear Factor κ B (NF κ B) activity. NF κ B is a critical pro-survival transcription factor in cancer initiation and progression with a prominent role in immortalization, angiogenesis and metastasis. We tested six curcumin analogs, which differ mainly in the number of phenol rings and nature of side groups for their inhibitory effect on *in vitro* proliferation and cell survival in a prostate cancer progression model featuring cell lineages with distinct hallmarks of prostate cancer progression. WST-1 proliferation assays conducted for time periods between 6 and 72 hours revealed a dose-dependent effect of the analogs within the 2-50 μ M range. Further, the WW49 and WW50 analogs showed anti-proliferative activity superior over curcumin with different activities in the different cells of the prostate cancer progression model. These lead compounds are currently being characterized for their toxicologic profiles including IC₅₀ determinations, and further tested for their NF κ B mediated inhibition of markers of prostate cancer progression (e.g. secretion of IL-6 and IL-8). Future work will include the testing and pharmacokinetic characterization in xenograft animal models of advanced prostate cancer for the most promising curcumin analogs. Thus, curcumin analogs may offer a novel alternative for prostate cancer therapy through the inhibition of cell survival pathways involving NF κ B, with promising potential as inhibitors of prostate carcinogenesis.