AD_____

Award Number: W81XWH-07-1-0158

TITLE: Mechanisms of Chinese Red Yeast Rice Inhibition of Prostate Cancer Growth

PRINCIPAL INVESTIGATOR: Mee Young Hong, PhD

CONTRACTING ORGANIZATION: University of California
Los Angeles, CA 90095

REPORT DATE: October 2009

TYPE OF REPORT: Final report

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY):*	**2. REPORT TYPE:**	**3. DATES COVERED** *(From - To)*
01-10-2009	Final report	*15 April 2007 – 14 September 2009*

4. TITLE AND SUBTITLE: Mechanisms of Chinese Red Yeast Rice Inhibition of Prostate Cancer Growth

5a. CONTRACT NUMBER

5b. GRANT NUMBER:
W81XWH-07-1-0158

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S) : Mee Young Hong

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of California, Los Angeles
Los Angeles, CA 90095

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
After publication, approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Prostate cancer is the second leading cause of cancer deaths in men in the United States. Early prostate cancer is androgen-dependent, but in later stages of the disease androgen-independent tumors arise with an eventual fatal outcome. Red Yeast Rice (RYR) is a traditional food spice consumed throughout Asia and contains a family of monacolins, one of which (monacolin K) is identical to lovastatin, with the ability to inhibit cholesterol synthesis. The objective of the study was to determine whether RYR can inhibit the growth of androgen-dependent and –independent prostate tumors in xenograft and to determine the underlying mechanisms. The study showed that RYR inhibited both androgen-dependent and androgen-independent xenograft prostate tumor volume by downregulation of gene expression involved in androgen synthesis (3β-hydroxysteroid dehydrogenase type 2, aldo-keto reductase family 1 member C3 and steroid 5α reductase type 1) and de novo cholesterol synthesizing enzyme (3-hydroxy-3-methyl-glutaryl CoA reductase) and its response element (sterol response element binding protein-2). RYR also reduced androgen receptor gene expression in androgen-independent xenograft. In gene profiles of microarray analysis, Many of genes were downregulated in RYR-fed androgen-dependent SCID tumors including androgen synthesis involved enzymes (AKR1C2, AKR1C3, AKR1B1 and AIG1) and ras and G-protein related genes (ARHGDIB, ARHGAP4, ARHGEF2, ARHGEF3, ARHGAP22, FAD5, FGD5, GIT2, GPR92, RHOBTB3, RGS2, RAC2, RIS and RICS). This study would establish a proof of principle that would strengthen the biological basis for human trials of RYR extract.

15. SUBJECT TERMS: Chinese Red Yeast Rice, prostate cancer, xenograft

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	UU	62	USAMRMC
					19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

Introduction

Prostate cancer is the second leading cause of cancer deaths in men in the United States (1). Chinese Red Yeast Rice (RYR) is a traditional food spice consumed throughout Asia (2, 3), and RYR contains a family of monacolins, one of which (monacolin K) is identical in structure to lovastatin, with the ability to inhibit cholesterol synthesis and lower plasma cholesterol levels in humans (4, 5). Since *de novo* cholesterogenesis is required for tumor growth, RYR may inhibit cancer cell growth. Statins are known to have anti-inflammatory properties (7, 8) and inflammation has been proposed as a critical step in prostate carcinogenesis. We hypothesized that RYR prevent against prostate cancer via cholesterol synthesis inhibition, inflammation or both. The primary specific aim of this proposal was to determine whether RYR can inhibit the growth of the androgen-dependent and –independent prostate tumors *in vivo*. A secondary specific aim was to determine the mechanisms by which RYR suppresses the growth of androgen dependent and androgen receptor-overexpressing androgen-independent LNCaP tumor xenografts.

Body

Task 1

Androgen-dependencey of LNCaP cells and androgen–independency of LNCaP-AR cells were established and the effects of RYR on human prostate cancer cell proliferation *in vitro* model data has been published in Journal of Medicinal Food 11:657- 666, 2008 (Title: Chinese red yeast rice vs lovastatin effects on prostate cancer cells with and without androgen receptor overexpression) (This paper is attached in appendix). Based on the *in vitro* study, androgen-dependent and –independent prostate cancer xenograft model was set up and the *in vivo* xenograft study as a function of RYR was carried out. RYR inhibited both androgen dependent LNCaP and androgen-independent LNCaP-AR xenograft tumor volume (Figure 1) (P<0.05).

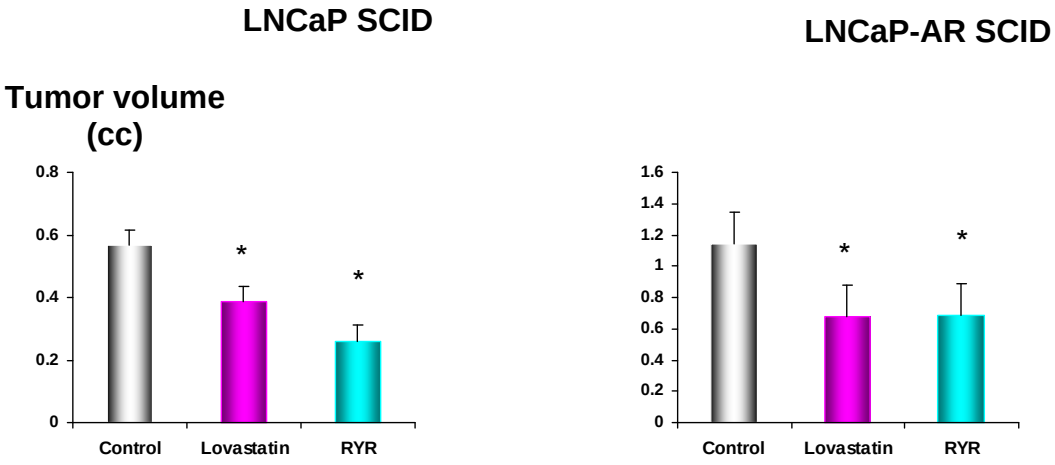


Figure 1. RYR inhibited androgen- dependent LNCaP and androgen-independent LNCaP-AR xenograft tumor volume (P<0.05).

Serum prostate specific antigen (PSA) levels were lower in RYR group compared to control group in LNCaP- AR xenografted animals (F igure 2) (P<0.05). The levels of monacolin K metabolite (lovas tatin hydroxyl acid) were measured in serum by high performance liquid chro matograph (HPLC) and they were the highest in RYR groups compared to lovastatin and control groups (Table 1).

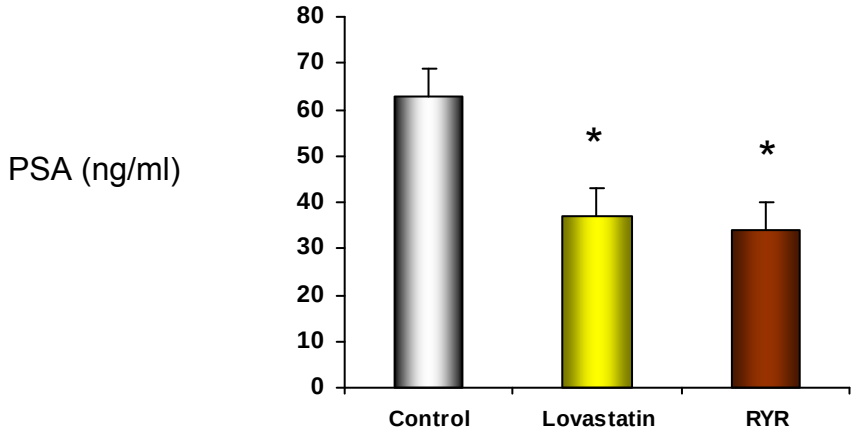


Figure 2. Serum prostate specific antigen (PSA) levels were lower in RYR group compared to control group in LNCaP-AR xenografted animals (P<0.05).

Table 1. Lovastatin hydroxyl acid level in serum

	Lovastatin hydroxy acid (ng/mL)
LNCaP Control	ND
LNCaP Drug	0.98
LNCaP RYR	**1.12**
LNCaP-AR Control	ND
LNCaP-AR Drug	0.19
LNCaP-AR RYR	**0.79**

ND: not detected

Task 2

RNAs of xenograft tumors we re extracted and the qualit ies wer e verified. Then microarray analysis using the tumor RNA was performed using illumina microarray. The data was analyz ed in UCL A Department of Medicine, Statistics Core. Many of genes were downregulated in RYR-fed androgen-dependent SCID tumors including androgen synthesis in volved enzy mes (AKR1C2, AKR1C3, AKR1B1 and AIG1) and ras and G-protei n related genes (ARHGDIB, ARHGAP4, ARHGEF2, ARHGEF 3, ARHGAP22, FA D5, FGD5, GIT2, GP R92, RHOBTB3, RGS2, RAC2, RIS and RICS). Some genes involved in cell proliferation (CCNA1, CCNB1IP1, CDCA7, GADD45 A, and GaK) and inflammation (IL8, IL23A and MAPK13) were decreased (Table 1). RYR group also dec reased ins ulin metabolism (IGF2BP2 and IRS2,) and signaling pathways (EMR2, MAP K13, SCAP1, SOCS2, TGFA and TNFSF10) related gene expression (Table 2).

Table 2. Genes expressed at lower levels in androgen-dependent prostate tumor of RYR group than control group

Gene name	Fold lower	Gene description
ADM	10	adrenomedullin
AKR1C3	10	aldo-keto reductase family 1
ARHGDIB	10	Rho GDP dissociation inhibitor
CAV1	10	caveolin
IL8	10	interleukin 8
ARL4	5	ADP-ribosylation factor-like 4
CA9	5	carbonic anhydrase
IGF2BP2	5	insulin-like growth factor 2
INPP1	5	inositol polyphosphate-1-phosphatase
LGALS3BP	5	lectin, galactoside-binding
NINJ2	5	ninjurin
NKD2	5	naked cuticle
SCAP1	5	src family associated phosphorprotein 1
ADAM19	3.3	metallopeptidase domain 19
AKR1C2	3.3	aldo-keto reductse family 1
ARHGAP4	3.3	Rho GTPase activaing protein
FGD5	3.3	RhoGEF and PH domain containing 5
FGF19	3.3	fibroblast growth factor 19
RGS2	3.3	regulator of G-protein signalling
SH3KBP1	3.3	SH3-domain kinase binding protein
AKR1B1	2.5	aldo-keto reductase fmialy 1
FYN	2.5	oncogene related to SRC, FGR
IL23A	2.5	interleukin 23A
IRS2	2.5	insulin receptor substrate
RAC2	2.5	ras-related C3 botulinum toxin substrate
TNFSF10	2.5	tumor necrosis factor receptor
ACACB	2	acetyl-Coenzyme A carboxylase beta
ACAD11	2	acyl-Coenzyme A dehydrogenase family
ARHGAP22	2	Rho GTPase activaing protein 22
ARHGEF3	2	Rho guanine nucleotide exchange factor
BCAR1	2	breast cancer anti-estrogen 1
BIK	2	BCL2-interacting killer
CCNA1	2	cyclin A1
CCNB1IP1	2	cyclin B1 interacting protein
CDCA7	2	cell division cycle associated 7
CTSB	2	cathepsin B
EMR2	2	egf-like module containing mucin-like hormone receptor-like
ERRFI1	2	ERBB receptor feedback inhibitor
GADD45A	2	growth arrest and DNA-damage inducible alpha
GPR92	2	G protein-coupled receptor

Table 2. Genes expressed at lower levels in androgen-dependent prostate tumor of RYR group than control group –continued

Gene name	Fold lower	Gene description
IL13RA1	2	interleukin 13 receptor, alpha
MAP3K7	2	mitogen-activated protein kinase
MAPK13	2	mitogen-activated protein kinase
PLD2	2	phospholipase D2
RHOBTB3	2	Rho-related BTB domain containing
RIS1	2	Ras-induced senescence 1
SOCS2	2	suppressor of cytokine signaling 2
STK24	2	serine/threonine kinase
TGFA	2	transforming growth factor, alpha
AIG1	1.4	androgen-induced 1
ARHGEF2	1.4	rho/rac guanine nucletide exchange factor
GAK	1.4	cyclin G associated kinase
GIT2	1.4	G protein-coupled receptor kinase interactor
RICS	1.4	Rho GTP-ase associated protein

RYR diet upregulated several genes incl uding cyclin-dependent kinase inhibitor 1A (CDKN1A), tumor protein p53 inducib le nuclear protein 1 (TP53INP1) and metallopeptidase (MMP7 and MMP10) in androgen-dependent SCID tumors (Table 3).

Table 3. Genes expressed at higher levels in androgen-dependent prostate tumor of RYR group than control group

Gene name	Fold higher	Gene description
MMP7	2.7	matrix metallopeptidase
CDKN1A	2.2	cyclin-dependent kinase inhibitor 1A
PDE3A	2	phosphodiesterase 3A
GDF15	1.7	growth differentiation factor 15
TP53INP1	1.7	tumor protein p53 inducible nuclear protein 1
ANXA5	1.6	annexin A5
MMP10	1.5	matrix metallopeptidase 10

Task 3

The gene expressions of androgen-independent tumor related genes (androgen receptor, 3 β-hydroxysteroid dehydrogenase type 2 (HSD3B2), aldo-keto reductase family 1 member C3 (AKR1C3), steroid 5α reductase type 1 (SRD5A1) were determined as a function of RYR diet. RYR downregulated HSD3B2, AKR1C3 and SRD5A1 genes for androgen synthes is more than two fold in both tumor xenografts ($p<0.05$) (Figure 3-5) . RYR downregulated androgen receptor gene expression only in androgen-independent LNCaP-AR xenografts ($P<0.05$) (Figure 6).

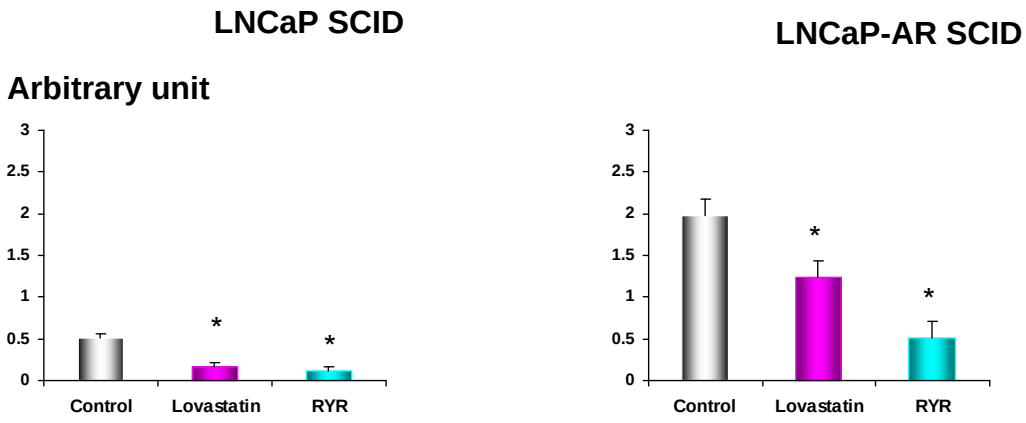


Figure 3. RYR downregulated 3 β-hydroxysteroid dehydrogenase type 2 (HSD3B2) gene expression in both LNCaP and LNCaP-AR xenografts($P<0.05$).

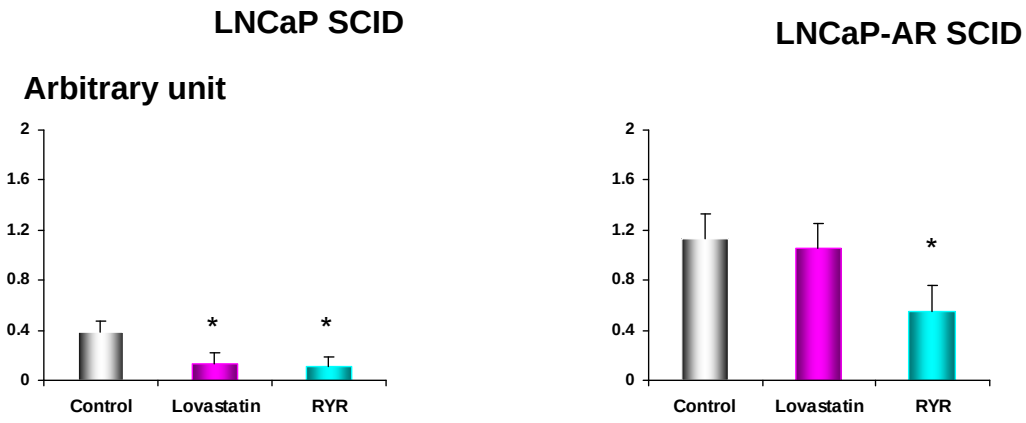


Figure 4. RYR downregulated aldo-keto reductase family 1 member C3 (AKR1C3) gene expression in both LNCaP and LNCaP-AR xenografts ($P<0.05$).

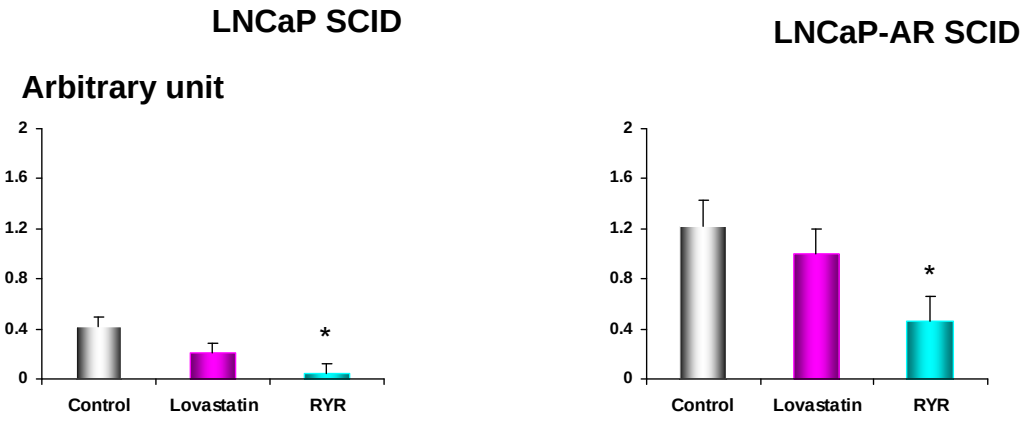


Figure 5. RYR downr egulated steroid 5 α reductase type 1 (SRD5A1) gene expression in both LNCaP and LNCaP-AR xenografts (P<0.05).

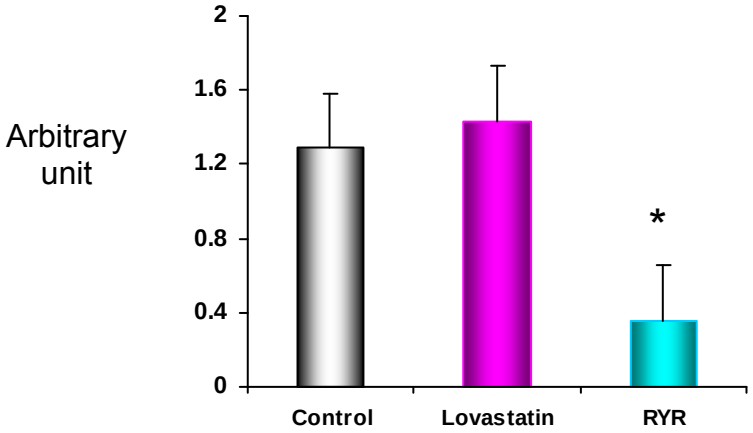


Figure 6. RYR downregulat ed androgen receptor (A R) gene expression in LNCaP-AR xenografts (P<0.05).

Task 4

It was investigated if the effect R YR inhibits the gene express ion involved in de novo cholesterogenes is. RYR downregulat ed 3-hydroxy-3-methyl-glutaryl CoA reductase (HMGCR) gene expression compared to control (P<0.05) (Figure 7).

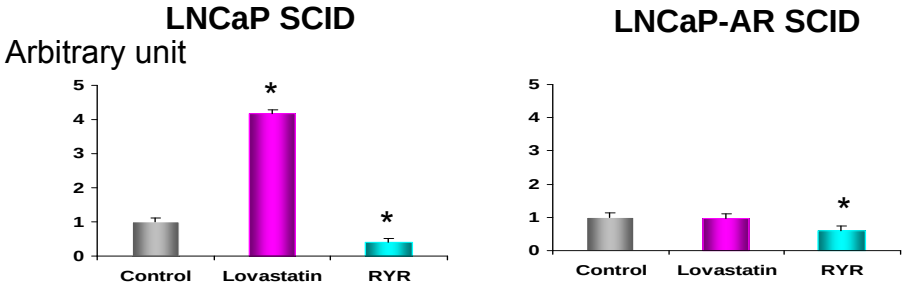


Figure 7. RYR downregulated HMGCR expression in both xenografts (P<0.05).

Task 5

RYR effects on gene expression involved in inflammation and oxidative s tress were also determined. RYR downregul ated RelA pro-inflammatory gene expression in both LNCaP and LNCaP-AR xenografts (P<0.05) (Figure 8). The oxidative stress and inflammation related genes (iNOS2, COX-2, 8-oxodeoxyguanosine, 8-oxoguanine-DNA glycosylase 1, glutathion e S-transferase, superoxide dismutase and catala se) were not significantly different among groups (data not shown).

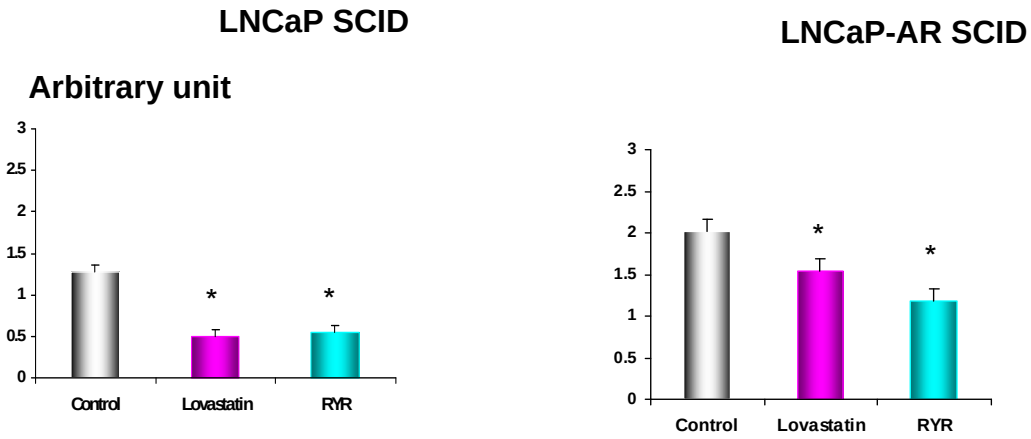


Figure 8. RYR downregulated RelA gene ex pression in both LNCaP and LNCaP-AR xenografts (P<0.05).

Task 6

One paper has been published in Journal of Medicinal Food and four abstracts have been submitted to scientific conf erences. Research data have been presentation in professional conferences. Another manuscript is in the final stage of preparation for journal submission.

Key research accomplishments

1. For the first time, this study showed RYR inhibited both androgen-dependent LNCaP and andr ogen-independent LN CaP-AR xenograft tumor volume.

2. This study showed RYR decreased serum prostate specific antigen (PSA) levels in LNCaP-AR xenografted animals.

3. Lovastatin (monacolin K) amount was measured in serum

4. This study demonstrated that RYR downregulated the gene expression of enzymes involved in androgen synthesis in both LNCaP and LNCaP-AR

xenografts: androgen 3β-hydroxysteroid dehydrogenase type 2 (HSD3B2), aldo-keto reductase family 1 me mber C3 (AKR1C3) and steroid 5 α reductase type 1 (SRD5A1).

5. This study illustrated that RY R downregu lated andr ogen rece ptor (AR) gene expression in LNCaP-AR xenografts.

6. This study also showed RYR down regulated the gene expre ssion of rate limit enzyme of cholesterol synthesis and the response element in both LNCaP and LNCaP-AR xen ografts: 3-hydroxy-3- methyl-glutaryl CoA reductase (HMGCR) gene expression in both LNCaP and LNCaP-AR xenografts ($P<0.05$).

7. The current microarray data show ed that many of genes were downregulated in RYR- fed androgen-dependent SCID tumors including androgen synthesis involved enzymes, ras and G-protein related genes , cell proliferation invo lving genes and inflammation related genes. RYR group also decreased insulin metabo lism and signaling pathways gene expression.

8. Parts of data of this study have been publis hed in a scientific peer reviewed journals.

9. Some dat a of this study has been presented in profe ssional national meetings.

10. The data have been used as prel iminary data for NIH RO3 grant (PAR-06-313) application.

Reportable outcomes

1) Paper publication

Mee Young Hong, Navindra P. S eeram, Yanjun Zhang and David Heber (2008) Chinese red yeast rice vs lovast atin effects on prostate cancer cells with and without androgen receptor overexpression Journal of Medicinal Food 11:657-666.

2) Abstract submission and research presentation

Mee Young Hong, Susanne Henning, Y anjun Zhang, Navindra P. Seeram, Aune Moro, and David Heber (2009) Chinese Red Yeast Rice inhibits tumor growth and downregulates expression of genes for androgen and cholesterol biosynthesis in human prostate cancer xenografts in S CID mice. The FASEB Journal 22.

3) Abstract submission and research presentation

M**ee Young Hong**, Aune Mor o, Yanjun Zhang, Navindra P. Seeram and David Heber (2008) Chinese red yeas t rice food spice inhibits androgen-dependent and –independent prostate tumor xenograft growth by inhibiting cholesterol biosynthesis in andr ogen-dependent and independent prostate cancer. The FASEB Journal 21.

4) Abstract submission and research presentation

M**ee Young Hong**, Navindra P. Seeram, Yanjun Zhang and David Heber (2007) Chinese red yeast rice ex tract inhibits androgen-dependent and –independent prostate cancer cell growth but by different mechanism. UCLA Department of Medicine Research conference

5) Paper preparation

Mee Young Hong, Susane Henning, Aune Moro, Navindra P. Seeram, Yanjun Zhang and David Heber Anticancer effe ct of Chinese red yeast rice on androgen-dependent and –i ndependent prostate cancer xenograft mice (final stage of preparation)

Conclusion

We were able to demonstrate that Chinese Red Yeas t Rice inhibits androgen-dependent and androgen-independe nt human prostate tumor growth by downregulation of genes involv ed in de novo choles terogenesis, inflammation, androgen synthesizing enzymes and androgen recept or. These results suggest the potential possibility that RYR is us ed as novel diet ary supplements with maximum potential for androgen-dependent and –independent prostate chemoprevention.

References

1. American Cancer Societ y. Cancer Facts and fi gures 2008. www.cancer.org. Atlanta, GA: American Cancer Society, 2008.
2. Wang HL, Fang SL: “Indigenous ferment ed foods of non-we stern origin” in Hesseltine CW, Wang HL (eds) Mcologia Memoirs pp. 317-344.
3. Simg R-H: “Tien-Kung K’ai-Wu” Chinese Technology in the Seventeent h Century. Pennsylvania State University Press. pp-292-294, 1966.
4. Li Z, Seeram NP, Lee R, Thames G, Minutti C, Wang HJ, Heber D. Plasma clearance of lovastatin versus chinese r ed yeast rice i n healthy volunteers. J. Altern. Complement. Med. 11:1031-1038, 2005.
5. Heber D, Lembertas A, Lu Q-Y, Bowe rman S, Go VLW. An analysis of nice proprietary Chines e Red Yeast Rice di etary supplements: Implications of variability in chemical profile and cont ents. J. Altern. Complement. Med. 7:133-139, 2001.
6. Bravi, F, Scotti, L, Bosetti C, Talami ni R, Negri E, Monte lla M, Franceschi S, La Vecchia C. Self-reported history of hypercholeste rolaemia and gallstones and the risk of prostate cancer. Ann. Onc. 2006 (in press).

7. Ridker PM, Rifai N, Pfeffer MA, Sacks FM, Moye LA, Goldman S, Flaker GC, Braunwald E. Inflammation, pravastatin, and the risk of coronary events after myocardial infarction in patients with average cholesterol levels. Cholesterol and Recurrent Events (CARE) Investigators. Circulation. 98:839-44, 1998.
8. Weitz-Schmidt G, Welzenbach K, Brinkmann V, Kamata T, Kallen J, Bruns C, Cottens S, Takada Y, Hommel U. Statins selectively inhibit leukocyte function antigen-1 by binding to a novel regulatory integrin site. Nat. Med. 7:687-92, 2001.

Appendices

1) Paper publication

Mee Young Hong, Navindra P. Seeram, Yanjun Zhang and David Heber (2008) Chinese red yeast rice vs lovastatin effects on prostate cancer cells with and without androgen receptor overexpression Journal of Medicinal Food 11:657-666.

Attached at the end of the appendices.

2) Abstract submission and research presentation

Mee Young Hong, Susanne Henning, Yanjun Zhang, Navindra P. Seeram, Aune Moro, and David Heber (2009) Chinese Red Yeast Rice inhibits tumor growth and downregulates expression of genes for androgen and cholesterol biosynthesis in human prostate cancer xenografts in SCID mice. The FASEB Journal 22.

Statin use has been associated with a reduced risk of prostate cancer. Chinese Red Yeast Rice (RYR) is a traditional food spice containing a family of eight monacolins one of which (monacolin K) is identical to lovastatin. The effects of 5% RYR in the diet on the growth of androgen-dependent (LNCaP) and androgen-independent (LNCaP-AR) prostate cancer xenografts in SCID mice over 8 wks was examined. The expression of genes regulating androgen biosynthesis and cholesterogenes is were determined by quantitative real time PCR. RYR inhibited both androgen-dependent and -independent prostate tumor xenograft growth by 54% and 40 %, respectively ($P<0.05$). RYR downregulated HSD3B2, AKR1C3 and SRD5A1 genes for androgen synthesis more than two fold in both tumor xenografts ($p<0.05$). RYR also downregulated HMGCR and SREBP-2 genes involved in de novo cholesterogenesis ($P<0.05$). RYR downregulated androgen receptor gene expression only in androgen-independent xenografts ($P<0.05$). Androgens are known to increase the growth of prostate cancer xenografts but this is the first study to demonstrate that RYR inhibits tumor growth and gene expression for both cholesterol biosynthesis and androgen biosynthesis. These studies demonstrate one of several possible pathways of RYR action in prostate cancer and suggest potential biomarkers for human studies. Supported by the UCLA CNRU CA 42710 and DOD W81XWH-07-1-0158.

3) Abstract submission and research presentation

Mee Young Hong, Aune Moro, Yanjun Zh ang, Navindra P. Seeram and David Heber (2008) Chinese red yeast rice food spice inhibits androgen-dependent and –independent prostate tumor xenograft growth by inhibiting cholesterol biosynthesis in androgen-dependent and independent prostate cancer. The FASEB Journal 21.

Large cohort studies demonstrate that users of cholesterol-lowering drugs have a reduced risk of prostate cancer. Xenogra ft studies demonstrate that statins can inhibit PCa xenograft growth by depleting lipid raft choles terol. Chinese Red Yeast Rice (RYR) is a food spice containing a family of monacolins one of which is identical to lovastatin (LV). We have prev iously demonstrated inhibition of cell proliferation with RYR treatment in human prostate cancer cells *in vitro*. The present study examined the effects of RYR on growth of androgen-dependent and androgen-independent human prostate cancer xen ografts in SCID mice. LNCaP, and androgen-independent LNCaP-AR cells were inoculated subcutaneously in mice receiving LV or 5% R YR diets over 8 w ks. RYR inhibit ed both androgen-dependent and –independent prostate tumor volume by more than 60 % in SCID mice ($P<0.01$). RYR showed a more potent effect in r educing tumor size than LV. RYR diet produced lower serum cholesterol and prostate specific antigen levels com pared to control and LV in LNCaP-AR xenograft mice ($P<0.05$). RYR food spice and nutritional strategies for lowe ring cholesterol are planned to explore the anti-cancer activity of RYR and s tatins in hum ans. Supported by UCLA CNRU CA 42710 and DOD W81XWH-07-1-0158.

4) Abstract submission and research presentation

Mee Young Hong, Navindra P. S eeram, Yanjun Zhang and David Heber **(**2007)Chinese red yeast rice extr act i nhibits androgen-dependent and –independent prostate cancer cell growth but by different mechanism. UCLA Department of Medicine Research conference

Early prostate cancer is androgen-dependent (AD), but in later stages of the disease androgen-independen t (AI) tumors arise with an eventual fatal outcome. RYR contains monacolin K (MK) which is id entical to lovastatin, with the ability to inhibit cholesterol synthesis. Since increas ed cholesterol level in prostate tissues is correlated with its maligna ncy, we hy pothesized that RYR may protect against prostate cancer by inhibiting canc er cell growth via downregulation of *de novo* cholesterogenesis. T wo human prostate canc er cell lines, either AD (LNCaP) or AI (LNCaP-AR), were tr eated with RYR or MK-free RY R. Cell proliferation and apoptosis were determined using the Cell Titer-Glo Luminescent viability assay and Cell Death Detection ELISA assay, re spectively. Transcription levels of 3-hydroxy-3-methyl-glutaryl CoA reductase (HMGCR) and sterol response ele ment binding pro tein-2 (SREBP-2) we re determi ned by real time PCR. RYR inhibited cell proliferation in both prostate c ancer cell lines ($p<0.001$) and stimulated apoptosis only in LNCaP cells ($p<0.01$). MK-free RYR showed similar results as the RYR treatment. Mevalonate (end produc t of HMGCR) treatment reversed the

RYR's anti-proliferative in LNCaP-AR c ells but not in LNCaP cells. RYR increased mRNA expression of HMGCR and SREBP-2 ($p<0.01$) but MK-free RYR decreased expression in both cell lin es ($p<0.01$). This study demonstrated that RYR inhibits cancer cell growth due to its MK component in AI prostate cancer cells, while via its other consti tuents and MK in AD cells. Funds: U CLA CNRU CA 42710 and DOD CDMRP PC060044.

5) Paper preparation

Mee Young Hong, Susane Henning, Aune Moro, Navindra P. Seeram, Yanjun Zhang and David Heber Anticancer effect of Chinese red yeast rice on androgen-dependent and –independent prostate cancer xenograft mice (final stage of preparation).

Attached at the end of the appendices.

JOURNAL OF MEDICINAL FOOD
J Med Food 11 (4) 2008, 657–666

DOI: 10.1089/jmf.2007.0702

Chinese Red Yeast Rice Versus Lovastatin Effects on Prostate Cancer Cells With and Without Androgen Receptor Overexpression

Mee Young Hong, Navindra P. Seeram, Yanjun Zhang, and David Heber

Center for Human Nutrition, David Geffen School of Medicine, University of California, Los Angeles, California

ABSTRACT Chinese red yeast rice (RYR), a food herb made by fermenting *Monascus purpureus* Went yeast on white rice, contains a mixture of eight different monacolins that inhibit cholesterogenesis and also red pigments with antioxidant properties. Monacolin K (MK) is identical to lovastatin (LV). Both LV and RYR contain statins, which could inhibit *de novo* cholesterogenesis, which is critical to the growth of tumor cells. Dysregulation of the cholesterol biosynthetic pathway has been demonstrated during progression to androgen independence in xenograft models, and it has been proposed that cholesterogenesis and androgen receptor (AR) up-regulation are essential to androgen-independent cell survival. This study was designed to examine the differences between the effects of RYR and LV on androgen-dependent LNCaP cells and androgen-independent cells overexpressing AR (LNCaP-AR). RYR showed more potent inhibition effect on prostate cancer cell growth compared to LV. Both the pigment and monacolin-enriched fractions purified from RYR inhibited proliferation ($P < .001$) to a lesser extent than intact RYR. While mevalonate, an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR), restored proliferation in LV-treated cells, it failed to do so in RYR-treated cells. Expression of the *HMGCR* gene was up-regulated by LV ($P < .001$) but not RYR in both LNCaP and LNCaP-AR cells. These results suggest that the RYR matrix beyond MK alone may be bioactive in inhibiting androgen-dependent and -independent prostate cancer growth. *In vivo* studies are needed to further establish the potential advantages of RYR over LV in prostate cancer chemoprevention and in the prevention of the emergence of androgen independence.

KEY WORDS: • ***Chinese red yeast rice*** • ***cholesterogenesis*** • ***3-hydroxy-3-methylglutaryl coenzyme A reductase*** • ***lovastatin*** • ***monacolins*** • ***pigment*** • ***prostate cancer***

INTRODUCTION

PROSTATE CANCER (PCa) is currently the most common malignancy in men in the United States, comprising 32% of all cancers and remains the second most common cause of cancer death in men in the United States, accounting for 11% of all cancer deaths.[1] The early stage of PCa is androgen-dependent and treatable.[2–7] However, after successful treatment, the emergence of androgen-independent PCa is common as the result of dysregulated gene expression leading to an adaptive up-regulation of cell survival genes, including the androgen receptor (AR).[3,4] These tumors are more difficult to treat, and they lead progressively to metastasis and death.[2–7] Therefore, novel approaches are needed to treat advanced androgen-independent PCa in order to reduce overall PCa mortality.

Chinese red yeast rice (RYR) is produced through solid-state fermentation of the yeast *Monascus purpeureus* Went. on white rice.[8–13] RYR contains predominantly rice starches and sugars, yeast polyketides (called monacolins), fatty acids, pigments, and condensed tannins.[12,13] The major monacolin found in RYR is monacolin K (MK), which is identical in structure to lovastatin (LV). Other polyketides in RYR are structural analogs of MK.[13] *Monascus* pigments comprise more than 10 compounds, six of which are well known: monascin, ankaflavin, monascorubin, rubropunctatin, monascorubramine, and rubropunctamine.[14–17] Recently, it has been reported that the pigments have antimicrobial[18,19] and anticancer[20–22] activities.

LV has been used throughout the world as a prescription cholesterol-lowering drug that can inhibit the enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR),[23–26] which forms mevalonate (MV), a key intermediate in the synthesis of cholesterol.

Our group conducted the first clinical trial of RYR in the United States.[27] A dose of 2,400 mg/day RYR, containing 0.4% by weight monacolins, resulted in an 18% decrease in

Manuscript received 6 December 2007. Revision accepted 11 March 2008.

Address reprint requests to Mee Young Hong, Ph.D. at her present address: School of Exercise and Nutritional Sciences, San Diego State University, 5500 Campanile Drive, San Diego, CA 92181-7251, E-mail: mhong2@mail.sdsu.edu

Address reprint requests to: David Heber, M.D., Ph.D., Center for Human Nutrition, University of California at Los Angeles, Los Angeles, CA 90095, E-mail: dheber@mednet.ucla.edu

total cholesterol, a 23% decrease in low-density lipoprotein cholesterol, and a 15% decrease in triglycerol concentrations.[27] *De novo* cholesterogenesis is required for tumor cell growth and for androgen synthesis. In fact, a recent case-control study reported that hypercholesterolemia was associated with a 50% increase in the risk of PCa,[28] and a growing body of evidence supports the notion that statins, including LV, may inhibit PCa cell growth in animal models and in humans.[29–32]

The present study was carried out to examine the differences between RYR and LV treatment on PCa cell growth in both the LNCaP cell and in the androgen-independent LNCaP-AR cell lines. The mechanism of action was studied by examining expression of the *HMGCR* gene and its transcription factor, sterol response element binding protein-2 (SREBP-2), following treatment with LV and RYR.

MATERIALS AND METHODS

Extract and standard preparation

Chinese RYR powder purchased from Botanica BioScience (Ojai, CA) was extracted with methylene chloride and evaporated under vacuum at 40°C. The MK concentration of the RYR extract was determined by high-performance liquid chromatography/mass spectrometry analysis (LCQ Classic Finnigan LC-MS/MS Systems, ThermoFinnigan, San Jose, CA) using an authentic standard (AG Scientific, San Diego, CA) as previously reported.[22] For MK-free RYR, endogenous MK in RYR was removed by injecting a sample of the RYR extract onto a Prep-LC 4000 system coupled with a model 490E Programmable Multiwavelength UV detector (Waters Corp., Milford, MA) with conditions as follows: column, Phenomenex (Torrance, CA) Sphereclone (250 × 21.2 mm × 10 mm), isocratic solvent system of methanol/water (8:2, vol/vol), flow 5 mL/minute, detection $\lambda = 237$ nm. To obtain the monacolin-rich fraction (MF-RYR) and pigment-rich fraction (PF-RYR) of RYR, the powdered RYR was extracted with a mixture of dichloromethane and acetone (1:1, vol/vol) solution and purified by silica-gel flash column chromatography, eluting with hexane and acetone (8:2, vol/vol), followed by pure acetone. The purified fractions—PF-RYR and MF-RYR—were 10% and 90% of RYR by weight, respectively.

Cell culture

The LNCaP human PCa cell lines was obtained from American Type Culture Collection (Rockville, MD), and LNCaP-AR cells were a generous gift from Dr. C. Sawyers (University of California Los Angeles, Los Angeles, CA). LNCaP and LNCaP-AR PCa cells were grown in RPMI 1640 medium, and the medium contained 10% fetal bovine serum (Life Technologies, Grand Island, NY) in the presence of 100 U/mL penicillin and 0.1 g/L streptomycin (Life Technologies). Cells were incubated at 37°C with 95% air and 5% CO_2. All cells were maintained below passage 20 and used in experiments during the linear phase of growth.

Cell proliferation assay

Proliferation was measured using the CellTiter-Glo Luminescent Cell Viability Assay (Promega, Madison, WI). When added to cells, the assay reagent produces luminescence in the presence of ATP from viable cells. Cells (5×10^3 per well) were seeded in 0.1 mL of the medium in sterile 96-well plates. After 24 hours, the medium was removed and replaced with treatment media. For the LV dose curve, cells were treated with LV (5. 93, 20, 40, or 80 μM) for 48 hours. The 5.93 μM LV is equivalent to the MK amount in 50 μg/mL RYR. For the RYR dose experiment, cells were treated with RYR (0–150 μg/mL) for 48 or 72 hours. To test the function of MK in RYR on PCa cell growth, cells were treated with MK-free RYR (0–100 μg/mL) for 48 hours. To compare the effect of whole RYR, MF-RYR, and PF-RYR on cell growth, cells were treated with RYR, MF-RYR (90% of RYR concentration), or PF-RYR (10% of RYR concentration) for 48 hours. MV (Sigma-Aldrich, St. Louis, MO) at 25 μM was used to test if the effect of RYR and its fraction is by *de novo* cholesterogenesis. All stock solutions of LV, RYR, MK-free RYR, MF-RYR, PF-RYR, and MV were dissolved in dimethyl sulfoxide (DMSO), and the final concentration of DMSO in medium was $<0.2\%$. At the end of treatment, plates were equilibrated, and then assay reagent was added to each well to induce cell lysis. The luminescence signal and results were read on an Orion Microplate Luminometer (Bertholds Detection Systems, Pforzheim, Germany). All plates had control wells containing medium without cells to obtain a value for background luminescence. Data are expressed as ratio to control (0.2% DMSO), and at least three independent experiments were replicated.

Apoptosis assay

Cells (10^5 per dish) were plated in 60-mm-diameter dishes for 24 hours, and then cells were treated with control (0.2% DMSO), LV (5.93 μM), RYR (50 μg/mL), or MK-free RYR (50 μg/mL) for 48 hours. Following treatments, apoptosis was assessed by measuring DNA fragmentation using the Cell Death Detection enzyme-linked immunosorbent assay ELISA^{PLUS} Assay (Roche, Indianapolis, IN) as previously described.[22] Two replicates per condition were assayed, and data averaged from three or four separate experiments are presented.

RNA extraction and reverse transcription (RT)

Total RNA was extracted using the RNeasy Mini Kit (Qiagen, Valencia, CA) and quantified by measuring the absorbance at 260 nm with a Gene Quant Spectrophotometer (Amersham-Pharmacia Biotech, Piscataway, NJ). RT was performed on 3 μg of RNA by using oligo$(dT)_{12–18}$ primers (Invitrogen, Carlsbad, CA) with SuperScript III reverse transcriptase (Invitrogen) according to the manufacturer's instructions.

Quantitative real-time polymerase chain reaction (PCR)

Expression of the genes for HMGCR and SREBP-2 was determined using Taqman Universal PCR master mix and

primers (Applied Biosystems, Foster City, CA) by quantitative real-time PCR using the ABI 7900 HT Sequence Detector (Applied Biosystems). The transcription levels of target genes were normalized to r18S expression. Some RT reaction repeated on a separate occasion, followed by PCR and quantitation to confirm the reproducibility of the assay. In addition, every set of RT reactions contains a without-RT negative control to confirm that no contamination or anomaly has occurred.

Statistics

Data for the proliferation, apoptosis, and gene expression were analyzed by Student's *t* test or one-way analysis of variance followed by Student-Newman-Keuls test with GraphPad PRISM version 3.0 (GraphPad Software, San Diego, CA).

RESULTS

Cell proliferation

Growth of the human PCa cell line LNCaP ($P < .01$) and LNCaP-AR ($P < .01$) cells was inhibited by LV in a dose-dependent manner at 48 hours (Fig. 1A). At a concentration of 5.93 μM, LV decreased prostate tumor cell growth by 20% and 15% in LNCaP and LNCaP-AR, respectively ($P < .01$) (Fig. 1A). Based on the chemical composition of RYR,

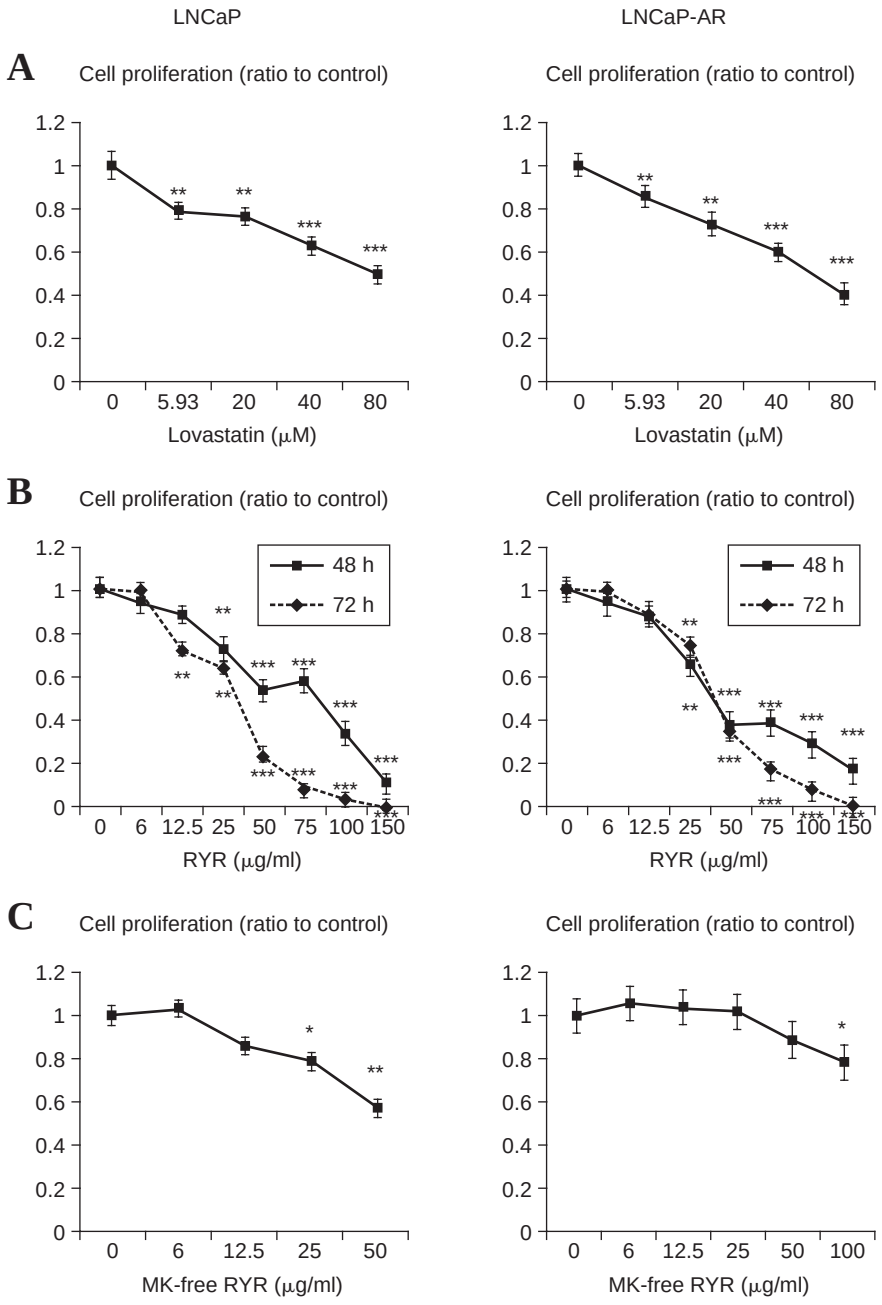


FIG. 1. LV and RYR effects on human PCa cell growth. (**A**) LV treatment for 48 hours decreased cell proliferation in a dose-dependent manner in LNCaP ($P < .01$) and LNCaP-AR ($P < .01$) human prostate cancer cells. (**B**) RYR decreased cell proliferation of both LNCaP and LNCaP-AR cells in a dose-dependent manner with 48-hour and 72-hour treatments ($P < .001$). (**C**) MK-free RYR treatment still decreased cell proliferation in LNCaP cells and LNCaP-AR cells ($P < .05$). Data are mean ± SEM values ($n = 3$–6). Significant differences from control (no treatment) are indicated: $^*P < .05$, $^{**}P < .01$, $^{***}P < .001$.

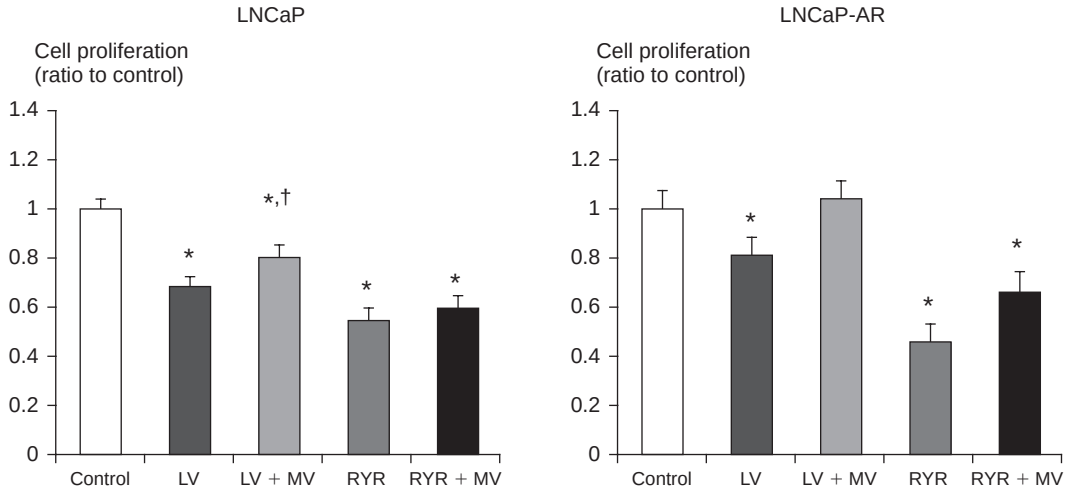


FIG. 2. MV effect on LV- or RYR-treated PCa cell growth. Addition of MV (25 μM) partially or fully abolished the antiproliferation activity of LV in LNCaP and in LNCaP-AR cells. Incubation with MV for 48 hours did not reverse the antiproliferative effect of RYR (50 μg/mL) in LNCaP and LNCaP-AR cells. Control contained 0.2% DMSO. Data are mean ± SEM values (n = 3–6). *Significantly different from control at $P < .05$. †Significantly different from LV at $P < .05$.

50 μg/mL RYR provides a concentration of 5.93 μM MK, and this led to 47% and 77% inhibition of LNCaP and 62% and 65% inhibition of LNCaP-AR cell growth in 48 and 72 hours of treatments, respectively ($P < .001$) (Fig. 1B). MK-free RYR treatment still decreased cell proliferation in LNCaP cells and LNCaP-AR cells ($P < .05$) (Fig. 1C). Addition of 25 μM MV to the medium of cells treated with 5.93 μM LV partially but significantly restored proliferation of LNCaP cells ($P < .05$) and fully in LNCaP-AR cells ($P < .05$) (Fig. 2). However, the same concentration of MV had no effect on the antiproliferative activity of RYR in LNCaP cells or LNCaP-AR cells (Fig. 2).

In order to determine which fraction of RYR exhibited the greatest antiproliferative potential, the effects of MF-RYR, PF-RYR, and RYR were compared on tumor cell growth. Both MF-RYR and PF-RYR inhibited cell growth in a dose-dependent manner in both LNCaP and LNCaP-AR PCa cells ($P < .001$) (Fig. 3). The two purified fractions obtained from RYR each only partially inhibited cell growth by comparison to intact RYR, which was more potent. In-

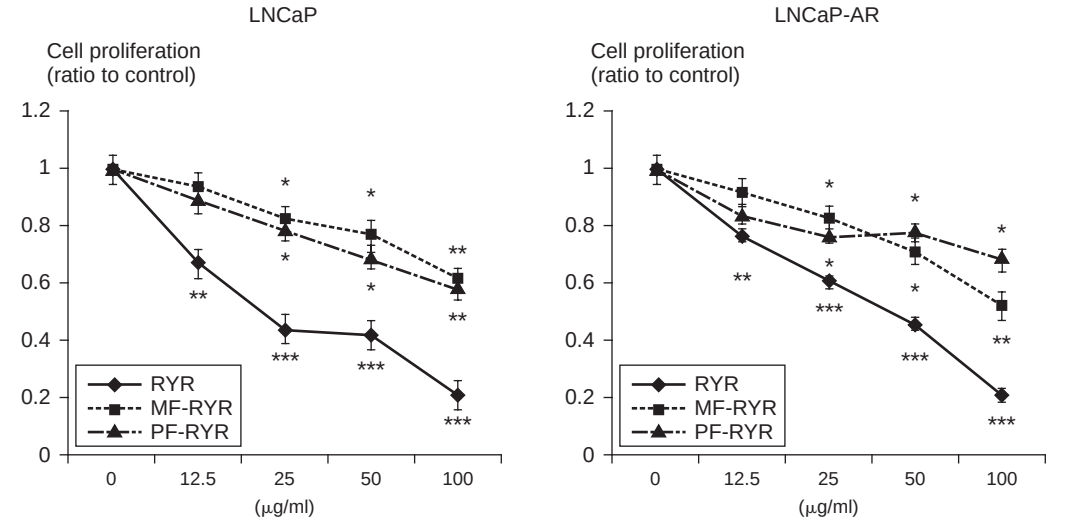


FIG. 3. Effect of PF-RYR, MF-RYR, or RYR effect on PCa growth. PF-RYRor MF-RYR treatment for 48 hours decreased cell proliferation in both LNCaP and LNCaP-AR cells ($P < .001$). However, the degree of antiproliferation was lower than that of RYR. Data are mean ± SEM values (n = 3–6). The proportions of PF-RYR and MF-RYR were 10% and 90% of RYR by weight, respectively. Therefore, for example, 50 μg/mL means 50 μg/mL RYR, 5 μg/mL PF-RYR, or 45 μg/mL MF-RYR. Significant differences from control (no treatment) are indicated: *$P < .05$, **$P < .01$, ***$P < .001$.

cubation with 25 μM MV partially abolished the antiproliferative effect of MF-RYR in both cells ($P < .05$) (Fig. 4A). In contrast, PF-RYR inhibited tumor cell growth regardless the MV treatment ($P < .05$) (Fig. 4B).

Apoptosis

The relative amount of induction of apoptosis was determined using an ELISA-based apoptosis assay, which quantitatively detects fragmented DNA. LV (5.93 μM) enhanced apoptosis in both LNCaP and LNCaP-AR cells by 1.7- and 2.1-fold, respectively ($P < .01$), and incubation with MV decreased the pro-apoptotic action of LV (Fig. 5A). Apoptosis was increased with RYR treatment at the level of 50 μg/mL in LNCaP ($P < .05$) (Fig. 5B). Incubation with MV did not decrease apoptosis in LNCaP cells treated with RYR (Fig. 5B). MK-free RYR also induced apoptosis in the LNCaP cells by more than 50% compared to controls ($P < .05$) but had no effect on the LNCaP-AR cells (Fig. 5C).

HMGCR and SREBP-2 gene expression

LV treatment up-regulated the expression of the *HMGCR* gene by more than fivefold in both LNCaP and LNCaP-AR prostate cancer cells ($P < .001$), but RYR did not (Fig. 6A). Cells treated with LV increased the expression of the *SREBP-2* gene by more than twofold in both LNCaP and LNCaP-AR cells ($P < .05$) (Fig. 6B). RYR treatment did not increase expression of the *SREBP-2* gene in LNCaP cells (Fig. 6B). In LNCaP-AR cells, RYR enhanced *SREBP-2* expression, but the increased amount was lower than that of LV ($P < .05$) (Fig. 6B).

DISCUSSION

Recent epidemiologic studies suggest a potential protective effect of statins in the patient against the risk of cancer at multiple sites, including the prostate.[33] While some studies show no effect of statins on PCa,[34,35] a recent, large cohort study showed a substantially reduced risk of metastatic or fatal PCa among statin users, with evidence of decreased risk with increasing duration of use.[36]

Cholesterol is a required intermediate in sex steroid synthesis, and reduction of testosterone precursors may influence the risk of progression and biology of PCa by suppressing steroid hormone production within the PCa cell. While several groups have shown that in men treated with

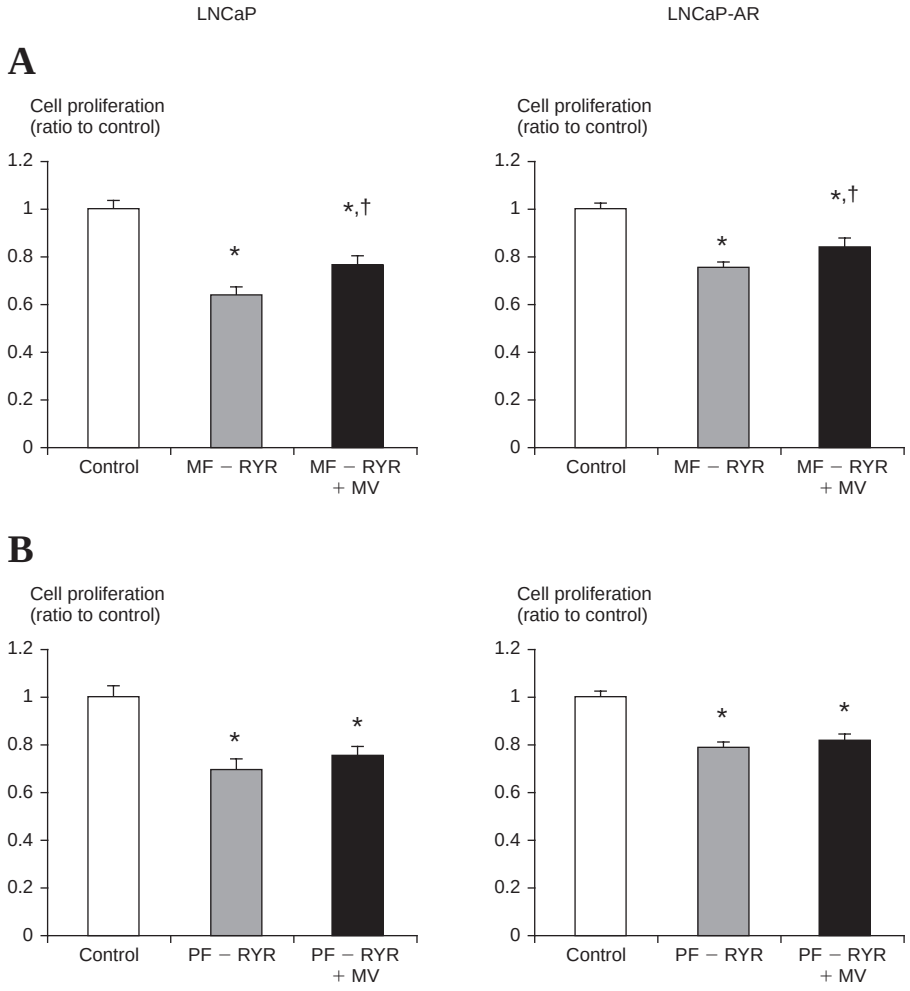


FIG. 4. MV effect on MF-RYR- or PF-RYR-treated PCa cell growth. (**A**) Incubation with 25 μM MV partially abolished the antiproliferative effect of MF-RYR in both cells. (**B**) In contrast, PF-RYR inhibited tumor cell growth regardless the MV treatment. Control contained 0.2% DMSO. Data are mean ± SEM values ($n = 3$–6). *Significantly different from control at $P < .05$. †Significantly different from MF-RYR at $P < .05$.

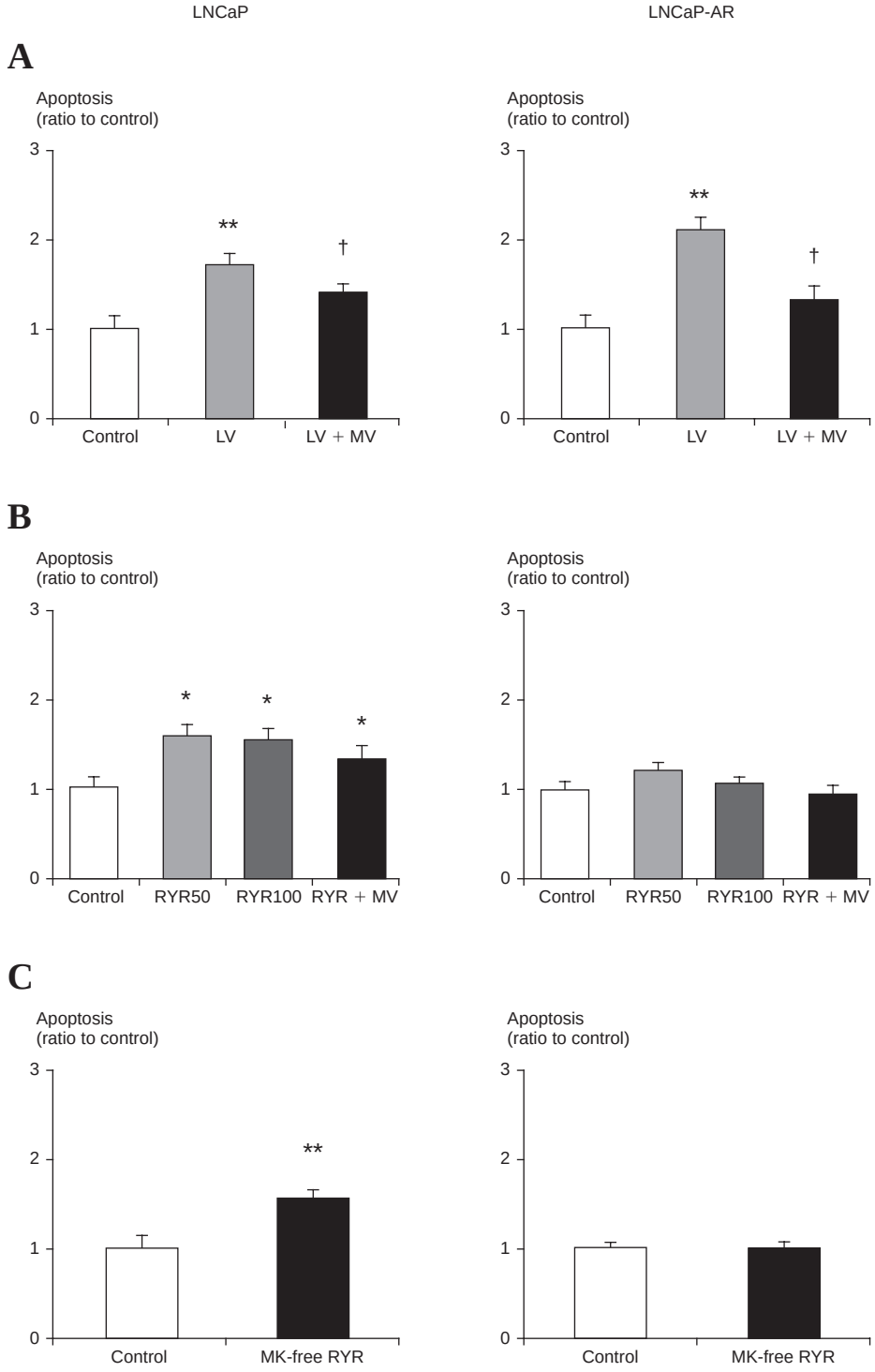


FIG. 5. Effects of LV, RYR, or MK-free RYR on apoptosis. (**A**) LV (5.93 μM) enhanced apoptosis ($P < .01$), and incubation with MV nullified the pro-apoptotic action of MK in both LNCaP and LNCaP-AR cells ($P < .05$). (**B**) RYR increased apoptosis regardless of the presence of MV in LNCaP cells ($P < .05$). In LNCaP-AR, there was no effect of RYR with and without treatment of MV. (**C**) MK-free RYR (50 μg/mL) enhanced apoptosis in LNCaP cells ($P < .01$). Control contained 0.2% DMSO. Data are mean ± SEM ($n = 3$–4). Significantly different from control at $^*P < .05$ or $^{**}P < .01$. †Significantly different from LV at $P < .05$.

agents that modulate serum testosterone, tissue androgen levels are relatively unchanged.[37–39] There may be effects of statins on intracellular androgen synthesis in the PCa cell. The mechanisms by which prostatic tissue maintains tissue androgens may include metabolism of adrenal androgens or *de novo* synthesis from cholesterol.[40] Statin may decrease androgen synthesis by reducing the precursor (*i.e.*, cholesterol) of androgen via inhibiting *de novo* cholesterolgenesis in prostate tissue. In patients undergoing androgen deprivation therapy to treat PCa, statins could influence disease progression via effects on residual androgen production, which might help explain the association between PCa progression and statin use.[36] Other mechanisms through which statins may influence PCa severity have also been proposed,[24] such

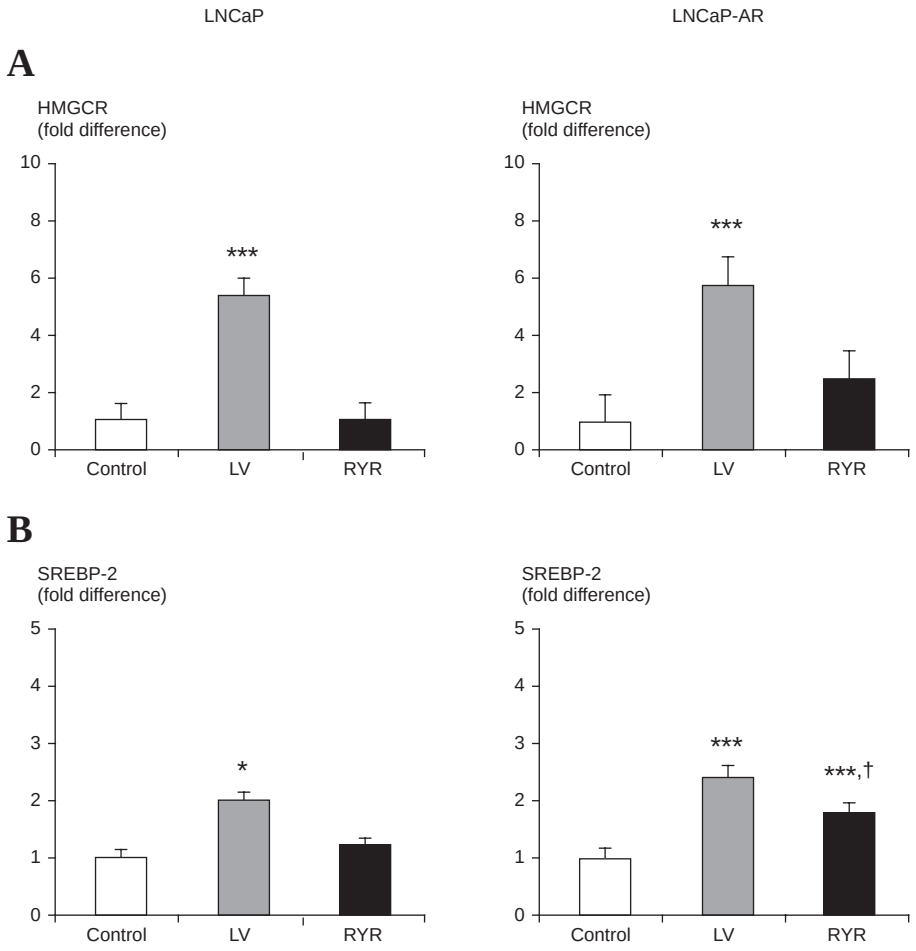


FIG. 6. LV and RYR effects on expression of the gene for (**A**) *HMGCR* and (**B**) *SREBP-2* in LNCaP and LNCaP-AR PCa cells. LV (5.93 μM) up-regulated *HMGCR* and *SREBP-2* gene expression in both LNCaP and LNCaP-AR cells. There was no significant effect of RYR (50 μg/mL) versus control on mRNA level of HMGCR in both cells. Control contained 0.2% DMSO. Data are mean ± SEM values (n = 3–6). Significantly different from control at $*P < .05$ or $***P < .001$. †Significantly different from LV at $P < .05$.

as decreasing prostate-specific antigen in small studies[41] and increasing prostate epithelial cell sensitivity to apoptosis.[42]

The AR has been implicated in the development and progression of recurrent PCa, and its expression is frequently up-regulated in androgen-independent PCa.[43–45] Although variation of expression of AR protein has been correlated with response to androgen deprivation therapy,[46,47] AR expression appears similar in androgen-dependent and recurrent PCa, but the receptor can be mutated.[48] When characterized functionally, most of the mutant ARs retain transcriptional activity in response to androgens, and some have altered steroid-binding specificity that changes the spectrum of ligands capable of activating androgen receptor.[49–51] Therefore, it is likely that up-regulation of the AR contributes to the emergence of androgen-independent PCa by enhancing the response to androgens in the circulation and those synthesized in the PCa cell.

De novo cholesterogenesis may be a key target for the prevention of the emergence of androgen-independent PCa. Much convincing evidence indicates that cells manifest a higher flux through the MV pathway when proliferating than when they are in the cell cycle arrest condition; furthermore, tumors undergo deregulated cholesterogenesis mainly at the critical rate-controlling juncture (*i.e.*, the reaction catalyzed by HMGCR). The MV component of the cholesterol biosynthesis plays a key role in controlling cell proliferation by generating prenyl intermediates, particularly farnesyl and geranyl-geranyl moieties.[52] These isoprenoids covalently modify and thus modulate the biological activity of signal transducing proteins. Therefore, depletion of MV may affect the processing and the transforming activities of growth signals in the prostate cell, androgen biosynthesis, and membrane cholesterol composition.

In the current study, RYR decreased HMGCR expression in both androgen-dependent and -independent PCa cells. However, there was a different effect of RYR on SREBP-2 expression in the two PCa cell lines: RYR increased SREBP-2 expression in LNCaP-AR but not in LNCaP cells. Since LNCaP-AR cells are androgen independent with overexpression of AR, this may be related to the differential expression of SREBP-2 in LNCaP-AR compared to LNCaP cells. However, we need further research to answer why RYR increased SREBP-2 expression in LNCaP-AR but not in LNCaP cells. SREBP-2 is one of the factors known to affect the transcription of *HMGCR*. Expression of the *HMGCR* gene was not induced with RYR treatment regardless of an-

drogen dependency. We need more study to determine how the androgen dependency/status affects the response in *SREBP-2* gene expression and, furthermore, RYR effects on the translation level of *SREBP-2* and *HMGCR*.

We have previously shown that a dose of 2,400 mg of RYR powder daily, containing 0.4% monacolins or 5–7.5 mg of MK, reduced cholesterol levels in hypercholesterolemic subjects to a degree that was equivalent to what is typically observed with 20 mg of LV.[27] This suggests that other constituents in the RYR matrix were bioactive beyond MK alone. In the present study, the contributions of MK within RYR and the elements in RYR other than MK were determined. For this purpose, the fraction of RYR without MK, a fraction rich in pigments, and a fraction rich in monacolins but absent of the pigments were prepared. Addition of 25 μM MV partly or fully reversed the antiproliferative and pro-apoptotic activity of LV. The selective reversal of LV-mediated inhibition of proliferation and increase of apoptosis as the result of MV supplementation is due to the restoration of the *de novo* cholesterogenesis metabolic pathway. On the other hand, the RYR effect on cell proliferation and apoptosis was not affected by addition of MV, even though RYR contained the same range of MK concentrations as the medium containing MK alone. Furthermore, MK-free RYR still inhibited cell proliferation. These data suggest that RYR has an effect on proliferation that is independent of the MK in RYR. A matrix with other structural analogs and other substances including pigments was able to inhibit PCa cell proliferation and stimulate apoptosis. While our studies clearly demonstrate that there are other factors beyond MK mediating some of the effects of RYR, further studies are needed to determine the effects of other active components in RYR, including sterols, isoflavones, and tannins, on PCa cell growth and apoptosis.

In the present study, RYR showed decreased cancer cell proliferation and induced apoptosis in LNCaP cells. It has been reported that LV reduced DNA synthesis by a significant induction of $p21^{WAF1/Cip1}$ protein expression in vascular smooth muscle cells,[53] which may, in part, explain the potential mechanism of RYR on inhibition of cancer cell growth. Simvastatin potentiates tumor necrosis factor α-induced apoptosis through the down-regulation of nuclear factor κB signaling pathway in squamous cell carcinoma SCC4 cells.[54] This study also showed that statin administration induces apoptosis by increase of caspase-3 and poly(ADP-ribose) polymerase cleavage in cancer cells. In another study, LV decreased AKT protein expression in SCC6 cells,[55] which suggests the involvement of phosphatidylinositol 3-kinase signaling on apoptosis induction. Therefore, RYR, which naturally contains LV, may enhance apoptosis via down-regulation of nuclear factor κB and phosphatidylinositol 3-kinase/AKT signaling as well as via induction of caspase-3 and poly(ADP-ribose) polymerase.

Interestingly, there was a difference in the apoptotic sensitivity in LNCaP cells compared to LNCaP-AR. RYR had no effect on apoptosis in the LNCaP-AR cells. We speculate that LNCaP-AR cells are an advanced type of PCa cells so that they may be resistant to the apoptotic process. RYR decreased advanced androgen-independent prostate tumor cell growth mainly by inhibition of cell proliferation rather than induction of apoptosis.

We have recently showed that food components can alter the expression of AR gene and genes involved in androgen synthesis.[56] Therefore, RYR may down-regulate gene expression of AR- and androgen-synthesizing enzymes, which contribute to attenuation of the risk of advanced PCa. The present study obviously supports the inhibition effect of RYR on growth of LNCaP-AR PCa cells, which indicates the potential use of RYR as an anticancer agent against advanced-stage PCa.

RYR, a traditional Chinese food herb and a modern dietary supplement, has demonstrated *in vitro* effects including stronger inhibition of tumor cell growth compared to LV treatment in human androgen-dependent and -independent prostate cancer cells. Furthermore, LV increased expression of the gene for HMGCR, while RYR did not. The advantage of using RYR over LV, which is a drug, is that RYR decreases the cholesterol level without elevation of expression of the gene for HMGCR. The multiple effects of RYR *in vitro* suggest that further investigations in animal models and ultimately in humans to confirm the anticancer activity of RYR are warranted.

ACKNOWLEDGMENTS

We thank Dr. Simin Liu for allowing us to use his real-time PCR equipment. This study was funded by UCLA/NCI Clinical Nutrition Research Unit grant CA 42710. This work was also funded by grant W81XWH-07-1-0158 from the Department of Defense (Principal Investigator: M.Y.H.).

AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

REFERENCES

1. American Cancer Society Cancer facts and figures 2007. http://www.cancer.org (accessed December 1, 2007).
2. Attard G, Sarker D, Reid A, Molife R, Parker C, de Bono JS: Improving the outcome of patients with castration-resistant prostate cancer through rational drug development. *Br J Cancer* 2006;95:767–774.
3. Chen CD, Welsbie DS, Tran C, Baek SH, Chen R, Vessella R, Rosenfeld MG, Sawyers CL: Molecular determinants of resistance to antiandrogen therapy. *Nat Med* 2004;10:33–39.
4. Kokontis JM, Hay N, Liao S: Progression of LNCaP prostate tumor cells during androgen deprivation: hormone-independent growth, repression of proliferation by androgen, and role for p27Kip1 in androgen-induced cell cycle arrest. *Mol Endocrinol* 1998;12:941–953.
5. Zhang L, Johnson M, Le KH, Sato M, Ilagan R, Iyer M, Gambhir SS, Wu L, Carey M: Interrogating androgen receptor function in recurrent prostate cancer. *Cancer Res* 2003;63:4552–4560.

6. Jariwala U, Prescott J, Jia L, Barski A, Pregizer S, Cogan JP, Arasheben A, Tilley WD, Scher HI, Gerald WL, Buchanan G, Coetzee GA, Frenkel B: Identification of novel androgen receptor target genes in prostate cancer. *Mol Cancer* 2007;6:39–53.
7. Kim D, Gregory CW, French FS, Smith GJ, Mohler JL: Androgen receptor expression and cellular proliferation during transition from androgen-dependent to recurrent growth after castration in the CWR22 prostate cancer xenograft. *Am J Pathol* 2002; 160:219–226.
8. Ma J, Li Y, Ye Q, Li J, Hua Y, Ju D, Zhang D, Cooper R, Chang M: Constituents of red yeast rice, a traditional Chinese food and medicine. *J Agric Food Chem* 2000;48:5220–5225
9. Haval RJ: Dietary supplement or drug? The case of cholestin. *Am J Clin Nutr* 1999;69:175–176.
10. Stuart MD: *Chinese Material Medica: Vegetable Kingdom*. Southern Materials Center, Taipai, Republic of China, 1979.
11. Went FAFC: *Monascus purpureus* le champignon de l'angquac une nouvelle thelebolee. *Ann Soc Nat Bot* 1895;8:1–17.
12. Martinkova L, Patakova-Juzlova P, Krent V, Kucerova Z, Havlicek V, Olsovsky P, Hovorka O, Rihova B, Vesely D, Vesela D, Ulrichova J, Prikrylova V: Biological activities of oligoketide pigments of *Monascus purpureus*. *Food Addit Contam* 1999; 16:15–24.
13. Heber D, Lembertas A, Lu Q-Y, Bowerman S, Go VLW: An analysis of nice proprietary Chinese Red Yeast Rice dietary supplements: implications of variability in chemical profile and contents. *J Altern Complement Med* 2001;7:133–139.
14. Chen FC, Manchand PS, Whalley WB: The chemistry of fungi. LXIV. The structure of monascin: the relative stereochemistry of the azaphilones. *J Chem Soc* 1971;21:3577–3579.
15. Manchand PS, Whally WB, Chen FC: Isolation and structure of ankaflavin. *Phytochemistry* 1973;12:2531–2532.
16. Kuromo M, Nakanishi K, Shindo K, Tada M: Biosynthesis of monascorubrin and monascoflavin. *Chem Pharm Bull (Tokyo)* 1963;11:358–362.
17. Hadfield JR, Holker JSE, Stanway DN: The biosynthesis of fungal metabolites. Part II. The β-oxo-lactone equivalences in rubropunctatin and monascorubrin. *J Chem Soc* 1967;19:751–755.
18. Kim C, Jung H, Kim YO, Shin CS: Antimicrobial activities of amino acid derivatives of *Monascus* pigments. *FEMS Microbiol Lett* 2006;264:117–124.
19. Journoud M, Jones PJ: Red yeast rice: a new hypolipidemic drug. *Life Sci* 2004;74:2675–2683.
20. Yasukawa K, Takahashi M, Natori S, Kawai K, Yamazaki M, Takeuchi M, Takido M: Azaphilones inhibit tumor promotion by 12-O-tetradecanoylphorbol-13-acetate in two-stage carcinogenesis in mice. *Oncology* 1994;51:108–112.
21. Yasukawa K, Takahashi M, Yamanouchi S, Takido M: Inhibitory effect of oral administration of *Monascus* pigment on tumor promotion in two-stage carcinogenesis in mouse skin. *Oncology* 1996;53:247–249.
22. Hong MY, Seeram NP, Zhang Y, Heber D: Anticancer effects of Chinese red yeast rice versus monacolin K alone on colon cancer cells. *J Nutr Biochem* 2008;19:448–458.
23. Retterstol K, Stugaard M, Gorbitz C, Ose L: Results of intensive long-term treatment of familial hypercholesterolemia. *Am J Cardiol* 1996;78:1369–1374.
24. Demierre MF, Higgins PD, Gruber SB, Hawk E, Lippman SM: Statins and cancer prevention. *Nat Rev Cancer* 2005; 5:930–942.
25. Lewis-Barned NJ, Ball MJ: Beneficial effect of simvastatin in patients with drug resistant familial hypercholesterolaemia. *N Z Med J* 1992;105:284–286.
26. Jenkins DJ, Kendall CS, Marchie A, Faulkner DA, Wond JM, de Souza R, Emam A, Parker TL, Vidgen E, Lapsley KG, Trautwein EA, Josse RG, Leiter LA, Connelly PW: Effects of a dietary portfolio of cholesterol-lowering foods vs lovastatin on serum lipids and C-reactive protein. *JAMA* 2003;290:502–510.
27. Heber D, Yip I, Ashley JM, Elashoff DA, Elashoff RM, Go VL: Cholesterol-lowering effects of a proprietary Chinese red-yeast-rice dietary supplement. *Am J Clin Nutr* 1999;69:231–236.
28. Shannon J, Tewoderos S, Garzotto M, Beer TM, Derenick R, Palma A, Farris PE: Statins and prostate cancer risk: a case-control study. *Am J Epidemiol* 2005;162:318–325.
29. Moyad MA, Merrick GS, Butler WM, Wallner KE, Galbreath RW, Kurko B, Adamovich E: Statins, especially atorvastatin, may favorably influence clinical presentation and biochemical progression-free survival after brachytherapy for clinically localized prostate cancer. *Urology* 2005;66:1150–1154.
30. Blais L, Desgagne A, LeLorier J: 3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibitors and the risk of cancer: a nested case-control study. *Arch Intern Med* 2000;160:2363–2368.
31. Marcelli M, Cunningham GR, Haidacher SJ, Padayatty SJ, Sturgis L, Kagan C, Denner L: Caspase-7 is activated during lovastatin-induced apoptosis of the prostate cancer cell line LNCaP. *Cancer Res* 1998;58:76–83.
32. Padayatty SJ, Marcelli M, Shao TC, Cunningham GR: Lovastatin-induced apoptosis in prostate stromal cells. *J Clin Endocrinol Metab* 1997;82:1434–1439.
33. Moorman PG, Hamilton RJ: Statins and cancer risk: what do we know and where do we go from here? *Epidemiology* 2007;18: 194–196.
34. Coogan PF, Rosenberg L, Strom BL: Statin use and the risk of 10 cancers. *Epidemiology* 2007;18:213–219.
35. Coogan PF, Rosenberg L, Palmer JR, Strom BL, Zauber AG, Shapiro S: Statin use and the risk of breast and prostate cancer. *Epidemiology* 2002;13:262–267.
36. Platz EA, Leitzmann MF, Visvanathan K, Rimm EB, Stampfer MJ, Willett WC, Giovannucci E: Statin drugs and risk of advanced prostate cancer. *J Natl Cancer Inst* 2006;98:1819–1825.
37. Marks LS, Mazer NA, Mostaghel E, Hess DL, Dorey FJ, Epstein JI, Veltri RW, Makarov DV, Partin AW, Bostwick DG, Macairan ML, Nelson PS: Effect of testosterone replacement therapy on prostate tissue in men with late-onset hypogonadism: a randomized controlled trial. *JAMA* 2006;296:2351–2361.
38. Mohler JL, Gregory CW, Ford OH III, Kim D, Weaver CM, Petrusz P, Wilson EM, French FS: The androgen axis in recurrent prostate cancer. *Clin Cancer Res* 2004;10:440–448.
39. Page ST, Lin DW, Mostaghel EA, Hess DL, True LD, Amory JK, Nelson PS, Matsumoto AM, Bremner WJ: Persistent intraprostatic androgen concentrations after medical castration in healthy men. *J Clin Endocrinol Metab* 2006;91:3850–3856.
40. Pelletier G, Luu-The V, El-Alfy M, Li S, Labrie F: Immunoelectron microscopic localization of 3β-hydroxysteroid dehydrogenase and type 5 17β-hydroxysteroid dehydrogenase in the human prostate and mammary gland. *J Mol Endocrinol* 2001;26:11–19.
41. Cyrus-David MS, Weinberg A, Thompson T, Kadmon D: The effect of statins on serum prostate specific antigen levels in a cohort of airline pilots: a preliminary report. *J Urol* 2005;173: 1923–1925.

42. Zhuang L, Kim J, Adam RM, Solomon KR, Freeman MR: Cholesterol targeting alters lipid raft composition and cell survival in prostate cancer cells and xenografts. *J Clin Invest* 2005; 115:959–968.
43. Feldman BJ, Feldman D: The development of androgen-independent prostate cancer. *Nat Rev* 2001;1:34–45.
44. Gelmann EP: Molecular biology of the androgen receptor. *J Clin Oncol* 2002;20:3001–3015.
45. Grossman ME, Huang H, Tindall DJ: Androgen receptor signaling in androgen refractory prostate cancer. *J Natl Cancer Inst* 2001;93:1687–1697.
46. Tilley WD, Lim-Tio SS, Horsfall DJ, Aspinall JO, Marshall VR, Skinner JM: Detection of discrete androgen receptor epitopes in prostate cancer by immunostaining: measurement by color video image analysis. *Cancer Res* 1994;54:4096–4102.
47. Prins GS, Sklarew RJ, Pertschuk LP: Image analysis of androgen receptor immuno-staining in prostate cancer accurately predicts response to hormonal therapy. *J Urol* 1998;159:641–649.
48. Visakorpi T, Hyytinen E, Koivisto P, Tanner M, Keinanen R, Palmberg C, Palotie A, Tammela T, Isola J, Kallioniemi OP: *In vivo* amplification of the androgen receptor geneand progression of human prostate cancer. *Nat Genet* 1995;9:401–406.
49. Tan J, Sharief Y, Hamil KG, Gregory CW, Zang DY, Sar M, Gumerlock PH, deVere White RW, Pretlow TG, Harris SE, Wilson EM, Mohler JL, French FS: Dehydroepiandrosterone activates mutant androgen receptors expressed in the androgen dependent human prostate cancer xenograft CWR22 and LNCaP cells. *Mol Endocrinol* 1997;11:450–459.
50. Peterziel H, Culig Z, Stober J, Hobisch A, Radmayr C, Bartsch G, Klocker H, Cato AC: Mutant androgen receptors in prostatic tumors distinguish between amino-acid-sequence requirements for transactivation and ligand binding. *Int J Cancer* 1995;63: 544–550.
51. Shi X, Ma A, Xia L, Kung H, de Vere White RW: Functional analysis of 44 mutant androgen receptors from human prostate cancer. *Cancer Res* 2002;62:1496–1502.
52. Graaf MR, Richel DJ, van Noorden CJ, Guchelaar HJ: Effects of statins and farnesyltransferase inhibitors on the development and progression of cancer. *Cancer Treat Rev* 2004;30:609–641.
53. Muller C, Kiehl MG, van de Loo J, Koch OM: Lovastatin induces p21WAF1/Cip1 in human vascular smooth muscle cells: influence on protein phosphorylation, cell cycle, induction of apoptosis, and growth inhibition. *Int J Mol Med* 1999;3:63–68.
54. Ahn KS, Sethi G, Aggarwal BB: Simvastatin potentiates TNF-alpha-induced apoptosis through the down-regulation of NF-kappaB-dependent antiapoptotic gene products: role of IkappaBalpha kinase and TGF-beta-activated kinase-1. *J Immunol* 2007;178: 2507–2516.
55. Mantha AJ, Hanson JEL, Goss G, Lagarde AE, Lorimer IA, Dimitroulakos J: Targeting the mevalonate pathway inhibits the function of the epidermal growth factor receptor. *Clin Cancer Res* 2005;11:2398–2407.
56. Hong MY, Seeram NP, Heber D: Pomegranate polyphenols down-regulate expression of androgen-synthesizing genes in human prostate cancer cells overexpressing the androgen receptor. *J Nutr Biochem* 2008 May 12 [Epub ahead of print].

Anticancer effect of Chinese red yeast rice on androgen-dependent and – independent prostate cancer xenograft mice

Mee Young Hong[1,2], Susane Henning[1], Aune Moro[1], Navindra P. Seeram[1], Yanjun Zhang[1], Jenny Kotlerman[3] and David Heber[1]

[1] Center for Human Nutrition, David Geffen School of Medicine, University of California, Los Angeles, CA 90095, USA

[2] Current address: School of Exercise and Nutritional Sciences, San Diego State University, San Diego, CA 92182, USA

[1] Department of Medicine Statistics Core, David Geffen School of Medicine, University of California, Los Angeles, CA 90024, USA

Running Title: Chinese Red Yeast Rice and Prostate Cancer

Keywords: Chinese red yeast rice, cholesterogenesis, 3-hydroxy-3-methyl-glutaryl CoA reductase, lovastatin, monacolins, prostate cancer, xenograft

Address reprint requests to:
Mee Young Hong, PhD.
Center for Human Nutrition
University of California at Los Angeles
Los Angeles, CA 90095
Tel.: 310 206 1987
Fax: 310 206 5264.
E-mail: myhong@mednet.ucla.edu

Abbreviations: AKR1C3: aldo-keto reductase family 1, member C3, AR: androgen receptor, ELISA: enzyme-linked immunosorbent assay, HMGCR: 3-hydroxy-3-methyl-glutaryl CoA reductase, HPLC: high performance liquid chromatography, HSD3B2: 3β-hydroxysteroid dehydrogenase type 2, LV: lovastatin, MK: monacolin K, MV: mevalonate, PCa: prostate cancer, PCR: polymerase chain reaction, PSA: prostate specific antigen, RT: reverse transcription, RYR: Chinese red yeast rice, SCID: severe combined immunodeficiency, SRD5A1: steroid 5α reductase type 1.

ABSTRACT

Early stage of prostate cancer (PCa) is androgen-dependent while later stage of this disease advanced to androgen-independent. It is very critical to reduce levels of androgens in treatment of PCa. Since cholesterol is a precursor of androgen hormone, reduction of the intermediate may influence the risk of progression and biology of PCa. Chinese Red Yeast Rice (RYR) is a spice herb and contains monacolin K which is identical to lovastatin (LV) with cholesterol lowering properties. Therefore, the hypothesis of the study was that RYR may reduce the growth of androgen dependent and androgen receptor (AR)-overexpressing androgen-independent prostate tumors in SCID xenograft mice by reducing cholesterol and androgen synthesis. RYR significantly reduced androgen-dependent and androgen-independent tumor volumes compared to control ($P<0.05$). Serum cholesterol levels and the HMGCR, key enzyme of cholesterogenesis, dropped with RYR diets in both SCID mice ($P<0.05$). The anti-cancer effects of RYR were more potent than those of LV. The current study clearly demonstrated a significant correlation between tumor volume and serum cholesterol ($P<0.001$). RYR administration downregulated androgen synthesizing enzymes (HSD3B2, AKR1C3 and SRD5A1) in both tumor types ($P<0.05$) and androgen receptor in androgen-independent tumors ($P<0.05$). Microarray data demonstrated that RYR modulated G protein and ras related signaling which may be resulted from the alteration of mevalonate levels and following isoprenoid signaling via lower de novo cholesterogenesis. These results suggest the potential possibility that RYR is used as novel dietary supplements with maximum potential for androgen-dependent and –independent prostate chemoprevention.

INTRODUCTION

Prostate cancer (PCa) is the second most common cause of cancer death in men in the United States today (1). It is estimated that 192,280 new cases occurred and 27,360 men died of prostate cancer in 2009 (1). Early stage of prostate cancer is androgen dependent and it can be effectively treated by androgen ablation therapy, radiation and/or surgery (2-7). However prostate tumors relapse and advance to an androgen-independent state where they progress in the absence of circulating testosterone leading to metastasis and death (2-7). Reducing the rate of emergence of androgen-independent cells in late stages of advanced prostate cancer is critical to reducing overall mortality from prostate cancer. Since diet may influence the emergence of prostate cancer after primary treatment and its progression to advanced disease, some traditional Chinese spices may be useful in its prevention and treatment.

Red Yeast Rice (RYR) is a traditional food spice consumed throughout Asia (8-11) and its food and medical value is believed to date back more than a thousand years. RYR contains a family of monacolins, one of which is monacolin K. It was revealed that monacolin K is identical to lovastatin (LV), with the ability to inhibit cholesterol synthesis and lower plasma cholesterol levels (12, 13). Our group conducted that a dose of 2400 mg/day RYR, containing 0.4% by weight monacolins, resulted in significant reduction of total cholesterol and LDL cholesterol (14).

A recent case-control study (15) reported that hypercholesterolemia was associated with a 50 % increase in the risk of prostate cancer. In clinical studies, statin showed the protective effect against prostate cancer (16-24) although its protective mechanism is not understood. Since RYR is a natural source of statin, RYR may inhibit prostate tumor growth. Recently, we have

demonstrated inhibition of cell growth for both LNCaP human prostate cancer cells in vitro and a LNCaP cell line which overexpresses the androgen receptor (LNCaP-AR) and is androgen-independent (25).

There are several enzymes which are involved in intracellular testosterone synthesis in the prostate cancer cell (26 - 30). HSD3B2 catalyzes the conversion of dehydroepi-androsterone (DHEA) to androstenedione (26, 27). In addition, AKR1C3 converts androstenedione to testosterone and increased amounts of AKR1C3 have been demonstrated in prostatic adenocarcinoma and carcinoma (28). Testosterone is converted to DHT by 5α-reductase (SRD5A1) (29). Since DHT has a higher affinity for AR than testosterone, it has been proposed that DHT is critical to prostate cancer development (27). Inhibitors of SRD5A1 such as finasteride reduce prostate size, and have been shown to reduce the development of prostate cancers by 25 percent but to increase the numbers of advanced cancers found (31, 32). Therefore, androgen synthesis involving enzymes may be critical for the prostate cancer development.

The present study conducted for the first time the effects of RYR on the growth of androgen-dependent and closely related androgen-independent human prostate cancer xenografts in severe combined immunodeficiency (SCID) mice. This study also examined the potential mechanisms of the RYR effects on androgen dependent and -independent prostate tumors by carrying out microarray whole gene profile analysis.

MATERIALS AND METHODS

SCID animals and diets

Sixty male SCID mice, aged 5 weeks, were purchased from (Taconic Farms Inc., Hudson, NY) and housed 5 mice per cage in a pathogen-free environment. After acclimation, all mice were implanted on shoulders with androgen-dependent LNCaP (ATCC, Rockville, MD) or androgen-independent LNCaP-AR (gift from Charles Sawyers, University of California, Los Angeles, CA) human prostate cancer cells (8×10^6) subcutaneously. Mice were provided with control, lovastatin or RYR diet. The control diet was modified AIN 93G diet (Dyets, Bethehem, PA) with 20% fat (20% soybean oil). RYR diet contains 5% of RYR powder (Botanica Bioscience, Ojai, CA) with the modified AIN93G diet. For lovastatin diet, lovastatin (Mylan pHarmaceuticals Inc., Morgantown, WV) was added to the control diet with the same amount that was detected in 5% RYR diet. The lovastatin amounts in lovastatin diet and 5%RYR were determined by HPLC and the amount was very similar (Figure 1).

Animal weight, food intake and tumor volume were measured weekly. The tumor volume was calculated using the formula: length x width x height x 0.5236 (33). At sacrifice, primary tumors were excised and blood was collected through cardiac puncture. The animal protocol was approved by Animal Care Committee in University of California, Los Angeles.

Serum cholesterol and PSA

Serum cholesterol concentrations were determined by cholesterol enzymatic methods using cholesterol standard (StanBio, Boerne, TX). Serum PSA was measured with PSA ELISA kit (Diagnostic Systems Laboratories, Webster, TX) according to the manufacturer's protocol.

In situ cell proliferation and apoptosis

Paraformaldehyde-fixed and paraffin-embedded tumor tissues were used to determine in situ cell proliferation and apoptosis analysis. Proliferating cells were detected by using monoclonal Ki-67 antibody (BD Biosciences, San Diego, CA) (34, 35). Total number of cells and stained proliferating cells were counted in 2 sub-squares in 4 x 4 grid in 10 microscopic areas. Data are expressed as proliferation index (%) which was calculated by the equation ((# of proliferating cells in one grid/total number of cells in one grid)*100)).

Apoptosis assay was based on terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-biotin nick end labeling (Millipore, Billerica, MA) (34). Apoptotic cells were counted in 15 microscopic fields and data are expressed in number of apoptotic cells/field.

Microarray

Total RNA of tumors were extracted using TRIZOL reagent (Invitrogen, Carlsbad, CA) and quantified by Agilent Bioanalyzer (Santa Clara, CA). The cDNA generation from the RNA and microarray analysis was performed using Illumina Bead Chip system (Illumina, San Diego, CA) in Southern California Genotyping Consortium at UCLA. The primary data from Illumina Bead Chips were collected using Illumina BeadStudio software and exported to Excel files. For quality control, detection P set to <0.01 was used as a cutoff. The data were further processed at UCLA Department of Medicine Statistics Core to identify the differentially expressed genes based on t-test and fold difference of the expression level.

Quantitative real time PCR

Extracted total RNA (3 μg) was reversely transcripted by using oligo(dT)$_{12-18}$ primers (Invitrogen) with SuperScript III Reverse Transcriptase (Invitrogen). Gene expressions were determined using Taqman Universal PCR master mix and primers (Applied Biosystems, Foster City, CA) by quantitative real time polymerase chain reaction (PCR) using the ABI 7900 HT Sequence Detector (Applied Biosystems) (36). The transcription levels of target genes were normalized to r18S expression. Every set of RT reactions contains a minus RT negative control to confirm that no contamination or anomaly has occurred.

Statistics

Data were analyzed by one-way ANOVA followed by Student-Newman-Keuls (SNK) multiple comparison with GraphPad PRISM 3.0 (GraphPad Software, San Diego, CA) in separate androgen-dependent and –independent SCID sets. The comparisons between LNCaP and LNCaP-AR were tested using t-test. The relationship between tumor volume and serum cholesterol was analyzed using correlation and linear regression model.

RESULTS

SCID Tumor volume

RYR significantly reduced androgen-dependent and androgen-independent tumor volumes compared to control by 54% and 41%, respectively ($P<0.05$) (Figure 2). Lovastatin also decreased tumor volume but only in androgen-dependent SCID animals by 32% ($P<0.05$) and lesser degree than those of RYR (Figure 2).

In situ cell proliferation and apoptosis

Proliferation index was lower in RYR group in both androgen-dependent and –independent SCID tumors by 32% and 47% ($P<0.05$) (Figure 3). There was no significant effect of RYR or lovastatin on apoptosis (data not shown).

Serum PSA

PSA is a risk marker of prostate cancer and often elevated in the presence of prostate cancer. The PSA level was higher in SCID animals compared to C57BL/6 mice (data not shown) and RYR decreased serum PSA levels compared to control in both LNCaP and LNCaP-AR injected SCID animals ($P<0.05$) (Figure 4).

Serum cholesterol and HMGCR gene expression

Serum cholesterol levels dropped with RYR diets by 20% and 30% in androgen-dependent and –independent SCID tumor ($P<0.05$) (Figure 5). Lovastatin drug which was same amount found in RYR diet decreased serum cholesterol by 10% in androgen-dependent SCID animals by not in androgen-independent SCID mice ($P<0.05$) (Figure 5). HMGCR gene expression was downregulated by 60% and 40% in RYR diet in both SCID animals ($P<0.05$) (Figure 6). In contrast, lovastatin drug increased four folds HMGCR gene expression compared to control diet (Figure 6). There was a strong correlation between tumor volume and serum cholesterol levels. As tumors were getting bigger, serum cholesterol increased or vise versa ($R^2=0.6571$, $P<0.001$) (Figure 7).

Gene expression of androgen synthesizing enzymes and androgen receptor (AR)

RYR-fed animals downregulated HSD3B2, AKR1C3 and SRD5A1 gene expression by more than three and two folds in LNCaP and LNCaP-AR SCID tumors, respectively ($P<0.05$) (Figure 8A, 8B and 8C). RYR diet also decreased AR expression by more than 2 folds in androgen-independent SCID tumors ($P<0.05$) (Figure 9). The transcription levels of HSD3B2, AKR1C3 and SRD5A1 and AR were higher in androgen-independent tumors than androgen-dependent tumors ($P<0.05$) (Figures 8 and 9).

Microarray

The analysis was focused on genes whose expression could most different between RYR group vs control in androgen-dependent and –independent tumors. Many of genes were downregulated in RYR-fed androgen-dependent SCID tumors including androgen synthesis involved enzymes (AKR1C2, AKR1C3, AKR1B1 and AIG1) and ras and G-protein related genes (ARHGDIB, ARHGAP4, ARHGEF2, ARHGEF3, ARHGAP22, FAD5, FGD5, GIT2, GPR92, RHOBTB3, RGS2, RAC2, RIS and RICS). Some genes involved in cell proliferation (CCNA1, CCNB1IP1, CDCA7, GADD45A, and GaK) and inflammation (IL8, IL23A and MAPK13) were decreased (Table 1). RYR group also decreased insulin metabolism (IGF2BP2 and IRS2,) and signaling pathways (EMR2, MAPK13, SCAP1, SOCS2, TGFA and TNFSF10) related gene expression (Table 1). FYN oncogene related SRC and FGR was downregulated by two folds in RYR treatment (Table 1).

RYR diet upregulated several genes including cyclin-dependent kinase inhibitor 1A (CDKN1A), tumor protein p53 inducible nuclear protein 1 (TP53INP1) and metallopeptidase (MMP7 and MMP10) in androgen-dependent SCID tumors (Table 2). In androgen-independent tumors, RYR diet decreased G-protein signaling genes (RGS2 and RICS), IL8, SOCS2 and CCNDBP1 (Table 3). The gene expressions of RGS2, RIS, ARHGEF2, RAC2 and MAPK13 in microarray data were confirmed by quantitative real time PCR analysis (Table 4).

DISCUSSION

Our study showed that RYR suppressed androgen-dependent and –independent prostate tumor formation in a SCID xenograft model. This result was also getting along with lower PSA levels and cell proliferation in both SCID tumors. Lower gene expressions of cell proliferation-related signaling in microarray data also support the finding. This is the first study to demonstrate the anticancer activity of RYR in human prostate tumor xenografts and the potential underlying mechanisms.

Androgens are critical to prostate cancer development as well as to the normal development, proliferation, and differentiation of prostate epithelial cells (2, 3). Androgen deprivation therapy has been main treatment for primary prostate cancer. However, research showed that, in men treated with agents that modulate serum testosterone, tissue androgen levels are relatively unchanged (37-39). This phenomenon may explain the recurrence of the PCa. At this stage, it is developed to androgen-independent which is fatal and metastatic. Therefore, it may be very critical to reduce levels of androgens in treatment of prostate cancer (2, 6, 40). Cholesterol is a required intermediate in steroid hormone (androgen and testosterone) synthesis,

and reduction of testosterone precursors may influence the risk of progression and biology of PCa by suppressing steroid hormone production within the PCa cell. The mechanisms by which prostatic tissue maintains tissue androgens may include metabolism of adrenal androgens or de novo synthesis from cholesterol (41, 42). The current study verified that greater cholesterol levels were strongly correlated with bigger prostate tumors. RYR downregulated enzymes involved in androgen synthesis (HSD3B2, AKR1C3 and SRD5A1) and reduced cholesterol level. RYR may decrease androgen synthesis by reducing the precursor (i.e. cholesterol) of androgen via inhibiting de novo cholesterogenesis and downregulation of androgen synthesizing enzyme genes. This is also supported in microarray results via downregulation of AKR1C2, AKR1C3, AKR1B1 and AIG1. In patients undergoing androgen deprivation therapy to treat PCa, RYR could influence disease progression via effects on residual androgen production.

Androgens' signaling occur via intracellular AR (2, 3, 43). The AR has been implicated in the development and progression of recurrent PCa and its expression is frequently upregulated in androgen-independent PCa (44-46). This contributes enhancement of the response to androgens in the circulation and those synthesized in the prostate cancer cell. Upregualtion of AR in androgen-independent tumor was consistent in our finding and our study found that RYR downregulated AR transcription levels in the androgen-independent SCID tumors. RYR may be particularly helpful in the subgroup of patients with androgen-independent PCa and AR upregulation.

De novo cholesterogenesis may be a key target for the prevention of the emergence of a PCa. Much convincing evidence indicates that tumors undergo deregulated cholesterogenesis mainly at the critical rate-controlling juncture (i.e., the reaction catalyzed by HMGCR). The

mevalonate component of the cholesterol biosynthesis plays a key role in controlling cell proliferation by generating prenyl intermediates, particularly farnesyl and geranyl-geranyl moieties (47-49). These isoprenoids covalently modify and thus modulate the biological activity of signal transducing proteins, such as G-protein involved signaling. Our microarray and quantitative real time PCR data demonstrated the relationship of RYR administration and downregulation of G-protein related gene expression such as ras oncogene signaling (50). It was shown in literature the linkage of AR singling with MAPK signal cascade and G-protein involvement (51). Reduction of mevalonate may affect the processing and the transforming activities of growth signals in the prostate cell, androgen biosynthesis, and membrane cholesterol composition.

We have previously shown the anticancer properties of RYR in human prostate cancer cell lines (25). The in vitro effects demonstrated that RYR showed stronger inhibition of tumor cell growth compared to LV treatment in human androgen-dependent and –independent prostate cancer cells. The results are extended in the current in vivo xenograft study. It is also interesting that LV administration enhanced HMGCR gene expression in androgen-dependent tumors but RYR downregulated the transcription levels in both types of tumor. The advantage of using RYR over LV, which is a drug, is that RYR reduced tumor volume, PSA level, cholesterol level without elevation of gene expression of HMGCR and gene expression related to androgen synthesis and inflammation.

RYR, a traditional Chinese food herb and a modern dietary supplement, has demonstrated inhibition of androgen-dependent and –independent prostate tumors. This implies the potential

use of RYR as novel dietary supplements with maximum potential for androgen-dependent and – independent prostate chemoprevention.

ACKNOWLEDGMENTS

We thank Dr. Simin Liu for allowing us to use his real time PCR equipment. This study was funded by UCLA/NCI Clinical Nutrition Research Unit Grant No. CA 42710. This work was also funded by W81XWH-07-1-0158 grant from Department of Defense (PI: MYH).

REFERENCES

1. American Cancer Society Cancer facts and figures 2009. www.cancer.org Atlanta, GA, 2009.
2. Attard G, Sarker D, Reid A, Molife R, Parker C, de Bono JS: Improving the outcome of patients with castration-resistant prostate cancer through rational drug development. *Br J Cancer* 2006;95:767-774.
3. Chen CD, Welsbie DS, Tran C, Baek SH, Chen R, Vessella R, Rosenfeld MG, Sawyers CL: Molecular determinants of resistance to antiandrogen therapy. *Nat Med* 2004;10:33-39.
4. Kokontis JM, Hay N, Liao S: Progression of LNCaP prostate tumor cells during androgen deprivation: hormone-independent growth, repression of proliferation by androgen, and role for p27Kip1 in androgen-induced cell cycle arrest. *Mol Endocrinol* 1998;12:941–953.
5. Zhang L, Johnson M, Le KH, Sato M, Ilagan R, Iyer M, Gambhir SS, Wu L, Carey M: Interrogating androgen receptor function in recurrent prostate cancer. *Cancer Res* 2003;63:4552–4560.
6. Jariwala U, Prescott J, Jia L, Barski A, Pregizer S, Cogan JP, Arasheben A, Tilley WD, Scher HI, Gerald WL, Buchanan G, Coetzee GA, Frenkel B: Identification of novel androgen receptor target genes in prostate cancer. *Mol Cancer* 2007;6:39-53.
7. Kim D, Gregory CW, French FS, Smith GJ, Mohler JL: Androgen receptor expression and cellular proliferation during transition from androgen-dependent to recurrent growth after castration in the CWR22 prostate cancer xenograft. *Am J Pathol* 2002;160:219-226.

8. Ma J, Li Y, Ye Q, Li J, Hua Y, Ju D, Zhang D, Cooper R, Chang M: Constituents of red yeast rice, a traditional Chinese food and medicine. *J Agric Food Chem* 2000;48:5220-5225

9. Wang HL, Fang SL: "Indigenous fermented foods of non-western origin" in Hesseltine CW, Wang HL (eds) Mcologia Memoirs pp 317-344, 1986.

10. Simg R-H: "Tien-Kung K'ai-Wu" Chinese Technology in the Seventeenth Century. Pennsylvania State University Press. pp 292-294, 1966.

11. Stuart MD: Chinese material medica: Vegetable kindom. Taipai, Republic of China: Southern materials Center, 1979.

12. Li Z, Seeram NP, Lee R, Thames G, Minutti C, Wang HJ, Heber D. Plasma clearance of lovastatin versus chinese red yeast rice in healthy volunteers. *J. Altern. Complement. Med.* 11:1031-1038, 2005.

13. Heber D, Lembertas A, Lu Q-Y, Bowerman S, Go VLW: An analysis of nice proprietary Chinese Red Yeast Rice dietary supplements: Implications of variability in chemical profile and contents. *J Altern Complement Med* 2001;7:133-139.

14. Heber D, Yip I, Ashley JM, Elashoff DA, Elashoff RM, Go VL: Cholesterol-lowering effects of a proprietary Chinese red-yeast-rice dietary supplement. *Am J Clin Nutr* 1999;69:231–236.

15. Bravi, F, Scotti, L, Bosetti C, Talamini R, Negri E, Montella M, Franceschi S, La Vecchia C. Self-reported history of hypercholesterolaemia and gallstones and the risk of prostate cancer. *Ann. Onc.* 2006;17:1014-1017.

16. Cyrus-David MS, Weinberg A, Thompson T, Kadmon D. The effect of statins on serum prostate specific antigen levels in a cohort of airline pilots: a preliminary report. J. Urol. 173:1923-1925, 2005.

17. Shannon J, Tewoderos S, Garzotto M, Beer TM, Derenick R, Palma A, Farris PE. Statins and prostate cancer risk: a case-control study. Am. J. Epidemiol. 162:318-325, 2005.

18. Moyad MA, Merrick GS, Butler WM, Wallner KE, Galbreath RW, Kurko B, Adamovich E. Statins, especially atorvastatin, may favorably influence clinical presentation and biochemical progression-free survival after brachytherapy for clinically localized prostate cancer. Urology. 66:1150-1154, 2005.

19. Marcelli M, Cunningham GR, Haidacher SJ, Padayatty SJ, Sturgis L, Kagan C, Denner L. Caspase-7 is activated during lovastatin-induced apoptosis of the prostate cancer cell line LNCaP. Cancer Res. 58:76-83, 1998.

20. Padayatty SJ, Marcelli M, Shao TC, Cunningham GR. Lovastatin-induced apoptosis in prostate stromal cells.J Clin Endocrinol Metab. 82:1434-1439, 1997.

21. Demierre MF, Higgins PD, Gruber SB, Hawk E, Lippman SM: Statins and cancer prevention. *Nat Rev Cancer* 2005;5:930-942.

22. Moorman PG, Hamilton RJ: Statins and cancer risk: what do we know and where do we go from here? *Epidemiology* 2007;18:194–196.

23. Coogan PF, Rosenberg L, Palmer JR, Strom BL, Zauber AG, Shapiro S: Statin use and the risk of breast and prostate cancer. *Epidemiology* 2002;13:262–267.

24. Platz EA, Leitzmann MF, Visvanathan K, Rimm EB, Stampfer MJ, Willett WC, Giovannucci E: Statin drugs and risk of advanced prostate cancer. *J Natl Cancer Inst* 2006;98:1819–1825.

25. Hong MY, Seeram NP, Zhang Y, Heber D. Chinese red yeast rice versus lovastatin effects on prostate cancer cells with and without androgen receptor overexpression. J Med Food. 2008;11:657-666.

26. Culig Z, Hoffmann J, Erdel M, Eder IE, Hobisch A, Hittmair A, Bartsch G, Utermann G, Schneider MR, Parczyk K, Klocker H. Switch from antagonist to agonist of the androgen receptor bicalutamide is associated with prostate tumour progression in a new model system. Br J Cancer 1999;81:242–51.

27. Devgan SA, Henderson BE, Yu MC, Shi CY, Pike MC, Ross RK, Reichardt JK. Genetic variation of 3 beta-hydroxysteroid dehydrogenase type II in three racial/ethnic groups: implications for prostate cancer risk. Prostate 1997;33::9-12.

28. Fung KM, Samara EN, Wong C, Metwalli A, Krlin R, Bane B, Liu CZ, Yang JT, Pitha JV, Culkin DJ, Kropp BP, Penning TM, Lin HK. Increased expression of type 2 3alpha-hydroxysteroid dehydrogenase/type 5 17beta-hydroxysteroid dehydrogenase (AKR1C3) and its relationship with androgen receptor in prostate carcinoma. Endocr Relat Cancer 2006;13:169-80.

29. Torres JM, Ruiz E, Ortega E. Development of a quantitative RT-PCR method to study 5alpha-reductase mRNA isozymes in rat prostate in different androgen status. Prostate 2003;56:74-9.

30. Stanbrough M, Bubley GJ, Ross K, Golub TR, Rubin MA, Penning TM, Febbo PG, Balk SP. Increased expression of genes converting adrenal androgens to testosterone in androgen-independent prostate cancer. Cancer Res 2006;66:2815-25.

31. Steers WD, 5alpha-reductase activity in the prostate. Urology 2001;58:17-24.

32. Thompson IM, Goodman PJ, Tangen CM, Lucia MS, Miller GJ, Ford LG, Lieber MM, Cespedes RD, Atkins JN, Lippman SM, Carlin SM, Ryan A, Szczepanek CM, Crowley JJ, Coltman CA. The Influence of Finasteride on the Development of Prostate Cancer N Engl J Med 2003;349:215-24.

33. Gleave, M.; Hsieh, J. T.; Gao, C. A.; von Eschenbach, A. C.; Chung, L. W. Acceleration of human prostate cancer growth in vivo by factors produced by prostate and bone fibroblasts *Cancer Res.* 1991;51:3753-3761.

34. Hong MY, Bancroft LK, Turner ND, Davidson LA, Murphy ME, Carroll RJ, Chapkin RS, Lupton JR. Fish oil decreases oxidative DNA damage by enhancing apoptosis in rat colon. Nutr Cancer. 2005;52:166-175.

35. Holt PR, Moss SF, Kapetanakis AM, Petrotos A, Wang S. Is Ki-67 a better proliferative marker in the colon than proliferating cell nuclear antigen? Cancer Epidemiol Biomarkers Prev. 1997;6:131-135.

36. Hong MY, Seeram NP, Zhang Y, Heber D: Anti-cancer effects of Chinese red yeast rice versus monacolin K alone on colon cancer cells. *J Nutr Biochem* 2008;19:448-458.

37. Marks LS, Mazer NA, Mostaghel E, Hess DL, Dorey FJ, Epstein JI, Veltri RW, Makarov DV, Partin AW, Bostwick DG, Macairan ML, Nelson PS: Effect of testosterone

replacement therapy on prostate tissue in men with late-onset hypogonadism: a randomized controlled trial. *JAMA* 2006;296:2351–2361.

38. Mohler JL, Gregory CW, Ford OH III, Kim D, Weaver CM, Petrusz P, Wilson EM, French FS: The androgen axis in recurrent prostate cancer. *Clin Cancer Res* 2004;10:440 -448.

39. Page ST, Lin DW, Mostaghel EA, Hess DL, True LD, Amory JK, Nelson PS, Matsumoto AM, Bremner WJ: Persistent intraprostatic androgen concentrations after medical castration in healthy men. *J Clin Endocrinol Metab* 2006;91:3850-3856.

40. Callewaert L, Van Tilborgh N, Claessens F. Interplay between two hormone-independent activation domains in the androgen receptor. Cancer Res. 2006;66:543-553.

41. Pelletier G, Luu-The V, El-Alfy M, Li S, Labrie F: Immunoelectron microscopic localization of 3ß-hydroxysteroid dehydrogenase and type 5 17ß-hydroxysteroid dehydrogenase in the human prostate and mammary gland. *J Mol Endocrinol* 2001;26:11–19.

42. Hong MY, Seeram NP, Heber D. Pomegranate polyphenols down-regulate expression of androgen-synthesizing genes in human prostate cancer cells overexpressing the androgen receptor. J Nutr Biochem. 2008;19:848-855.

43. Wang Q, Li W, Zhang Y, Yuan X, Xu K, Yu J, Chen Z, Beroukhim R, Wang H, Lupien M, Wu T, Regan MM, Meyer CA, Carroll JS, Manrai AK, Jänne OA, Balk SP, Mehra R, Han B, Chinnaiyan AM, Rubin MA, True L, Fiorentino M, Fiore C, Loda M, Kantoff PW, Liu XS, Brown M. Androgen receptor regulates a distinct transcription program in androgen-independent prostate cancer. Cell. 2009;138:245-256.

44. Feldman BJ, Feldman D: The development of androgen-independent prostate cancer. *Nature Rev* 2001;1:34-45.

45. Gelmann EP: Molecular biology of the androgen receptor. *J Clin Oncol* 2002;20:3001-3015.

46. Grossman ME, Huang H, Tindall DJ: Androgen receptor signaling in androgen refractory prostate cancer. *J Natl Cancer Inst* 2001;93:1687-1697.

47. Graaf MR, Richel DJ, van Noorden CJ, Guchelaar HJ: Effects of statins and farnesyltransferase inhibitors on the development and progression of cancer. *Cancer Treat Rev* 2004;30:609-641.

48. Mo H, Elson CE. Studies of the isoprenoid-mediated inhibition of mevalonate synthesis applied to cancer chemotherapy and chemoprevention. Exp Biol Med (Maywood). 2004;229:567-585.

49. Zhuang L, Kim J, Adam RM, Solomon KR, Freeman MR: Cholesterol targeting alters lipid raft composition and cell survival in prostate cancer cells and xenografts. *J Clin Invest* 2005;115:959–968.

50. Erlich S, Tal-Or P, Liebling R, Blum R, Karunagaran D, Kloog Y, Pinkas-Kramarski R. Ras inhibition results in growth arrest and death of androgen-dependent and androgen-independent prostate cancer cells. Biochem Pharmacol. 2006;72:427-436.

51. Heinlein CA, Chang C. The roles of androgen receptors and androgen-binding proteins in nongenomic androgen actions. Mol Endocrinol. 2002;16:2181-2187.

Figure legends

Figure 1. HPLC chromatograms of monacolin K in lovastatin diet (A) and 5% RYR diet (B). The amounts of lovastatin were very similar.

Figure 2. RYR effects on SCID tumor volume. RYR-fed mice significantly reduced androgen-dependent and androgen-independent tumor compared to control diet-fed mice. Values are Mean ± SE. *: Significantly different from control at $P<0.05$.

Figure 3. RYR effects on in situ cell proliferation. Proliferation index was lower in RYR group in both androgen-dependent and –independent SCID tumors Values are Mean ± SE. *: Significantly different from control at $P<0.05$.

Figure 4. RYR effects on PSA levels. RYR administration reduced serum PSA levels compared to control in both types of SCID mice. Values are Mean ± SE. *: Significantly different from control at $P<0.05$.

Figure 5. RYR effects on cholesterol levels. RYR reduced serum cholesterol levels more than 15% of control in androgen-dependent and –independent SCID mice. Values are Mean ± SE. *: Significantly different from control at $P<0.05$.

Figure 6. RYR effects on HMGCR gene expression. RYR downregulated HMGCR gene expression in both types of xenografted tumors. In contrast, lovastatin drug increased HMGCR gene expression in LNCaP xenograft animal. Values are Mean ± SE. *: Significantly different from control at $P<0.05$.

Figure 7. Correlation between tumor volume and serum cholesterol levels. As tumors were getting bigger, serum cholesterol increased or vise versa ($R^2=0.6571$, $P<0.001$). All data were combined.

Figure 8. RYR effects on gene expression involved in androgen synthesis. RYR-fed animals downregulated HSD3B2 (A), AKR1C3 (B) and SRD5A1 (C) gene expression by more than three and two folds in LNCaP and LNCaP-AR SCID tumors. Values are Mean ± SE. *: Significantly different from control at $P<0.05$.

Figure 9. RYR effects on gene expression of androgen receptor (AR). RYR diet also decreased AR expression by more than 2 folds in androgen-independent SCID tumors. Values are Mean ± SE. *: Significantly different from control at $P<0.05$.

Table 1. Genes expressed at lower levels in androgen-dependent prostate tumor of RYR group than control group

Gene name	Fold lower	Gene description
ADM	10	adrenomedullin
AKR1C3	10	aldo-keto reductase family 1
ARHGDIB	10	Rho GDP dissociation inhibitor
CAV1	10	caveolin
IL8	10	interleukin 8
ARL4	5	ADP-ribosylation factor-like 4
CA9	5	carbonic anhydrase
IGF2BP2	5	insulin-like growth factor 2
INPP1	5	inositol polyphosphate-1-phosphatase
LGALS3BP	5	lectin, galactoside-binding
NINJ2	5	ninjurin
NKD2	5	naked cuticle
SCAP1	5	src family associated phosphorprotein 1
ADAM19	3.3	metallopeptidase domain 19
AKR1C2	3.3	aldo-keto reductse family 1
ARHGAP4	3.3	Rho GTPase activaing protein
FGD5	3.3	RhoGEF and PH domain containing 5
FGF19	3.3	fibroblast growth factor 19
RGS2	3.3	regulator of G-protein signalling
SH3KBP1	3.3	SH3-domain kinase binding protein
AKR1B1	2.5	aldo-keto reductase fmialy 1
FYN	2.5	oncogene related to SRC, FGR
IL23A	2.5	interleukin 23A
IRS2	2.5	insulin receptor substrate
RAC2	2.5	ras-related C3 botulinum toxin substrate
TNFSF10	2.5	tumor necrosis factor receptor
ACACB	2	acetyl-Coenzyme A carboxylase beta
ACAD11	2	acyl-Coenzyme A dehydrogenase family
ARHGAP22	2	Rho GTPase activaing protein 22
ARHGEF3	2	Rho guanine nucleotide exchange factor
BCAR1	2	breast cancer anti-estrogen 1
BIK	2	BCL2-interacting killer
CCNA1	2	cyclin A1
CCNB1IP1	2	cyclin B1 interacting protein
CDCA7	2	cell division cycle associated 7
CTSB	2	cathepsin B
EMR2	2	egf-like module containing mucin-like hormone receptor-like
ERRFI1	2	ERBB receptor feedback inhibitor
GADD45A	2	growth arrest and DNA-damage inducible alpha
GPR92	2	G protein-coupled receptor

Table 1. Genes expressed at lower levels in androgen-dependent prostate tumor of RYR group than control group -continued

Gene name	Fold lower	Gene description
IL13RA1	2	interleukin 13 receptor, alpha
MAP3K7	2	mitogen-activated protein kinase
MAPK13	2	mitogen-activated protein kinase
PLD2	2	phospholipase D2
RHOBTB3	2	Rho-related BTB domain containing
RIS1	2	Ras-induced senescence 1
SOCS2	2	suppressor of cytokine signaling 2
STK24	2	serine/threonine kinase
TGFA	2	transforming growth factor, alpha
AIG1	1.4	androgen-induced 1
ARHGEF2	1.4	rho/rac guanine nucletide exchange factor
GAK	1.4	cyclin G associated kinase
GIT2	1.4	G protein-coupled receptor kinase interactor
RICS	1.4	Rho GTP-ase associated protein

Table 2. Genes expressed at higher levels in androgen-dependent prostate tumor of RYR group than control group

Gene name	Fold higher	Gene description
MMP7	2.7	matrix metallopeptidase
CDKN1A	2.2	cyclin-dependent kinase inhibitor 1A
PDE3A	2	phosphodiesterase 3A
GDF15	1.7	growth differentiation factor 15
TP53INP1	1.7	tumor protein p53 inducible nuclear protein 1
ANXA5	1.6	annexin A5
MMP10	1.5	matrix metallopeptidase 10

Table 3. Differential genes expression in androgen-independent prostate tumor of RYR group compared to control group

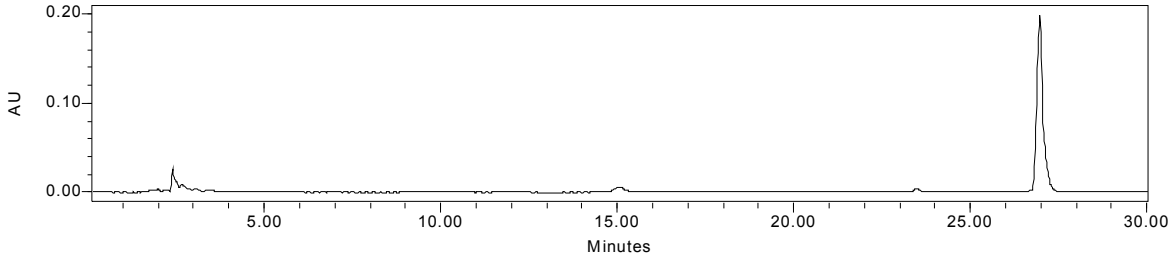
Gene name	Fold lower	Gene description
RGS2	2	regulator of G-protein signaling
SOCS2	2	suppressor of cytokine signaling 2
RICS	1.8	Rho GTPase-activating protein
IL8	1.6	interleukin 8
CCNDBP1	1.4	cyclin D binding protein

Gene name	Fold higher	Gene description
CDC14B	2.2	CDC14 cell division cycle 14 B
GDF15	1.4	growth differentiation factor 15

Table 4. Expression of RGS2, ARHGEF2, RAC2 and MAPK13 by quantitative real time PCR in LNCaP and LNCaP-AR SCID tumors

Gene name	LNCaP SCID (Fold lower)	LNCaP-AR SCID (Fold lower)
RGS2	2.6	NS
RIS	2.3	NS
ARHGEF2	1.9	1.5
RAC2	1.7	NS
MAPK13	1.7	NS
RelA	2.3	1.7

(A)


0.20
0.10
0.00
AU
5.00
10.00
15.00
20.00
25.00
30.00
Minutes

(B)

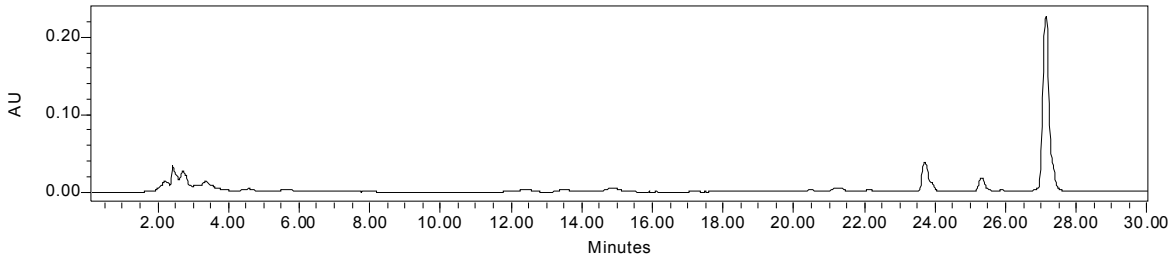

0.20
0.10
0.00
AU
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
26.00
28.00
30.00
Minutes

Figure 1

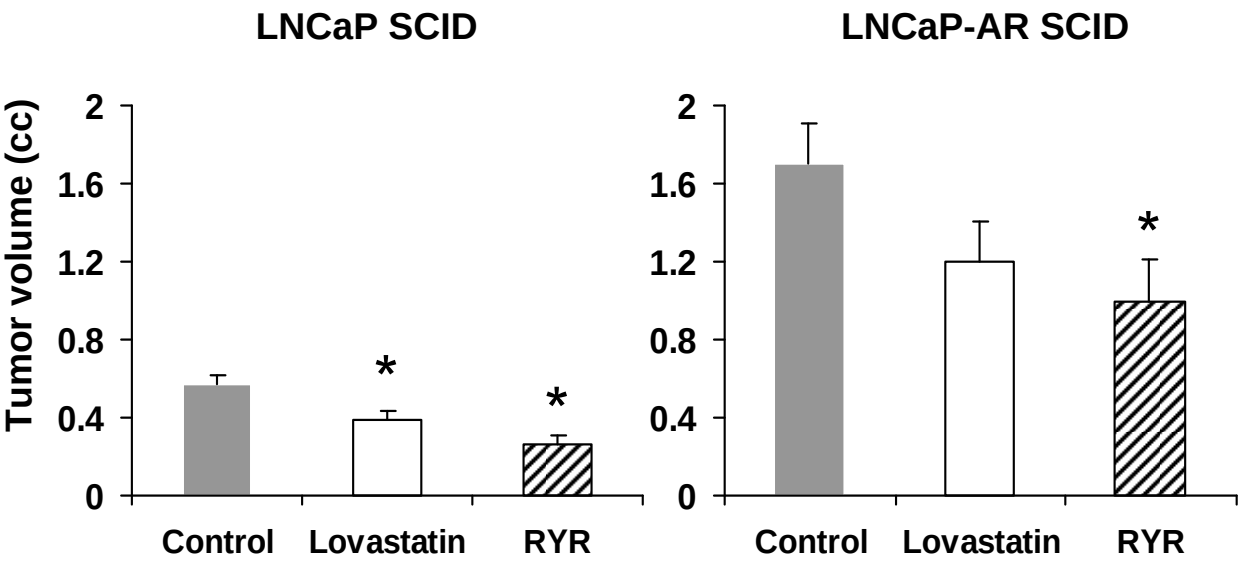

LNCaP SCID
LNCaP-AR SCID
Tumor volume (cc)
2
1.6
1.2
0.8
0.4
0
*
*
Control
Lovastatin
RYR
Control
Lovastatin
RYR

Figure 2

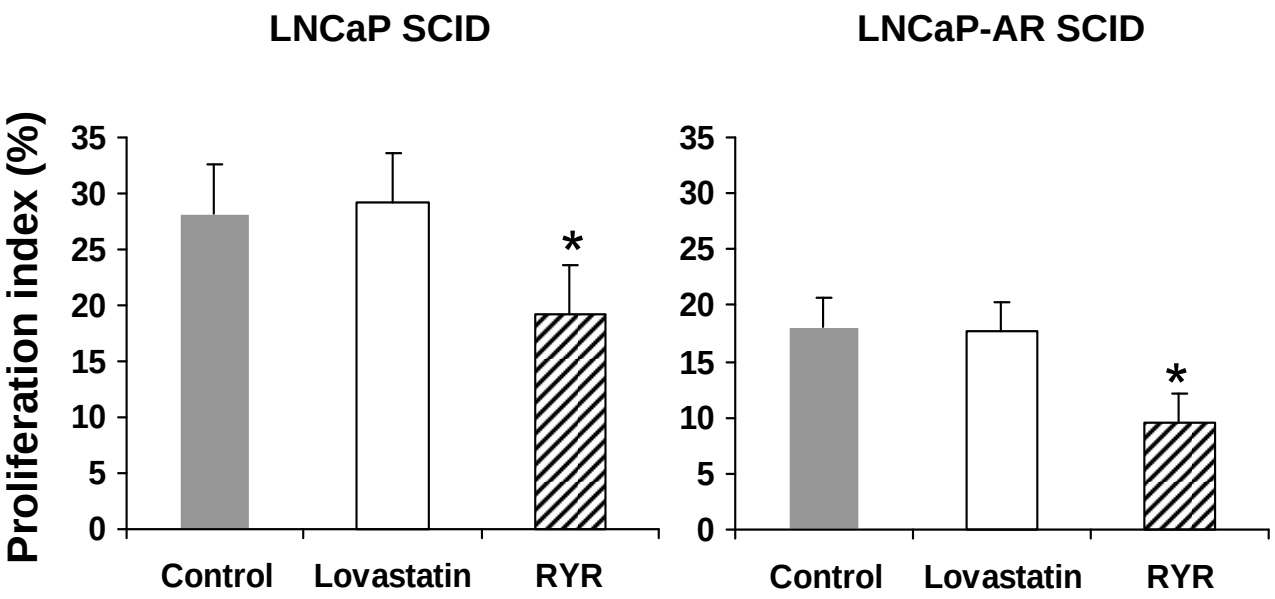

LNCaP SCID
LNCaP-AR SCID
Proliferation index (%)
35
30
25
20
15
10
5
0
*
*
Control
Lovastatin
RYR
Control
Lovastatin
RYR

Figure 3

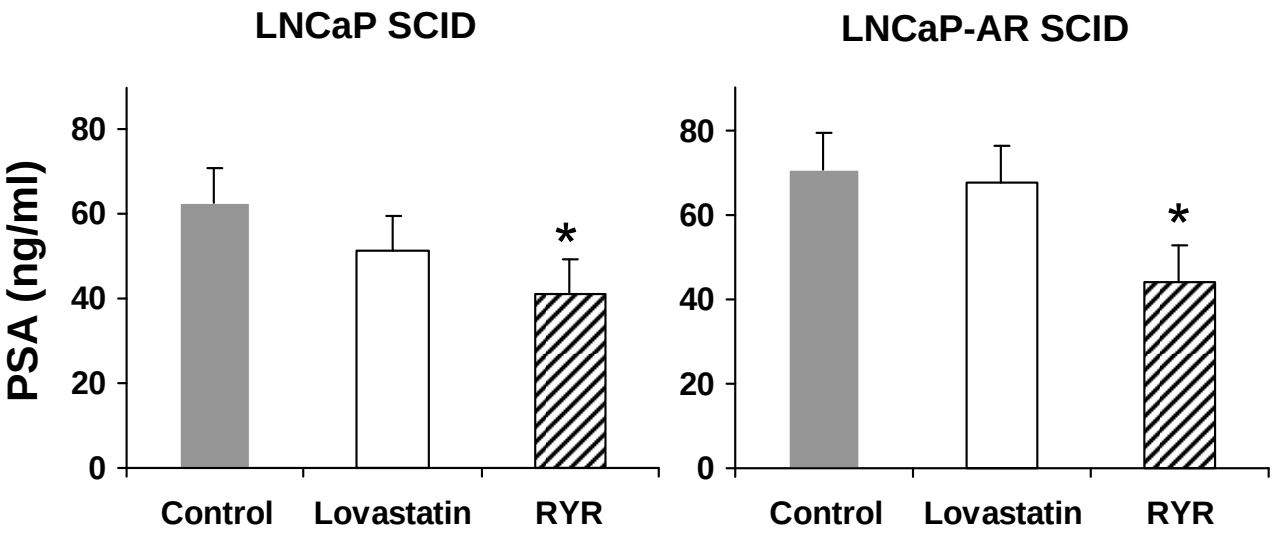

LNCaP SCID
LNCaP-AR SCID
PSA (ng/ml)
80
60
40
20
0
*
*
Control
Lovastatin
RYR
Control
Lovastatin
RYR

Figure 4

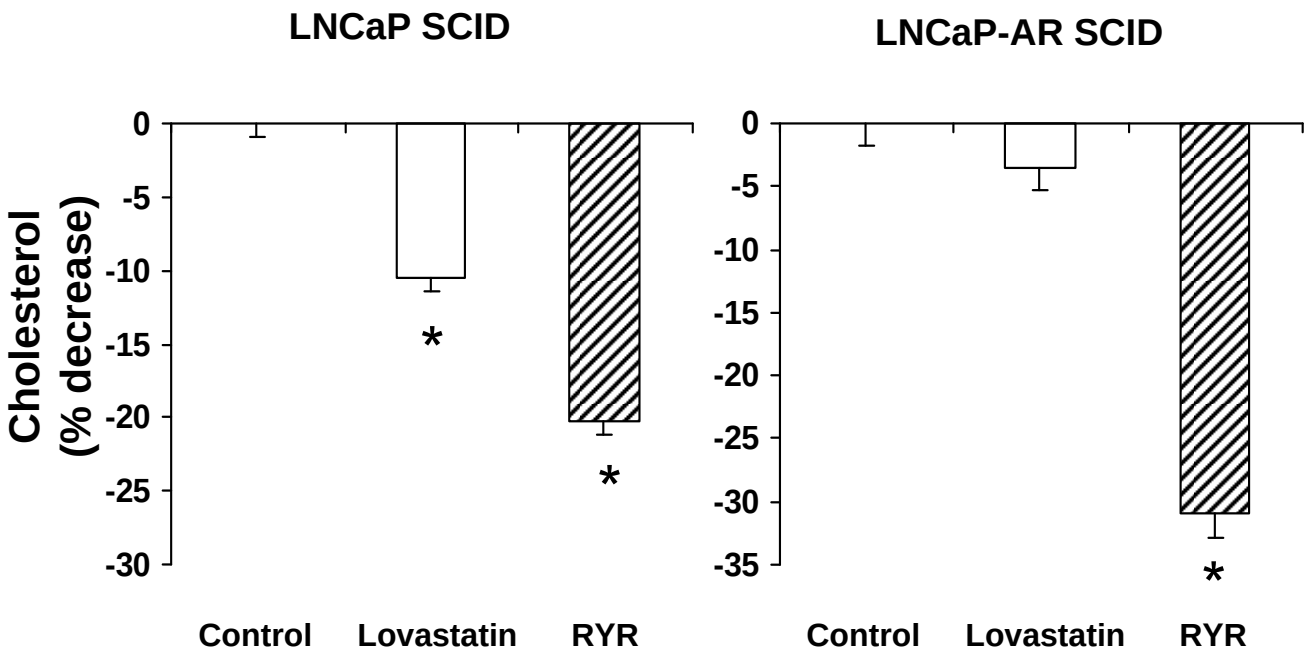

LNCaP SCID
LNCaP-AR SCID
Cholesterol (% decrease)
0
-5
-10
-15
-20
-25
-30
-35
*
Control
Lovastatin
RYR

Figure 5

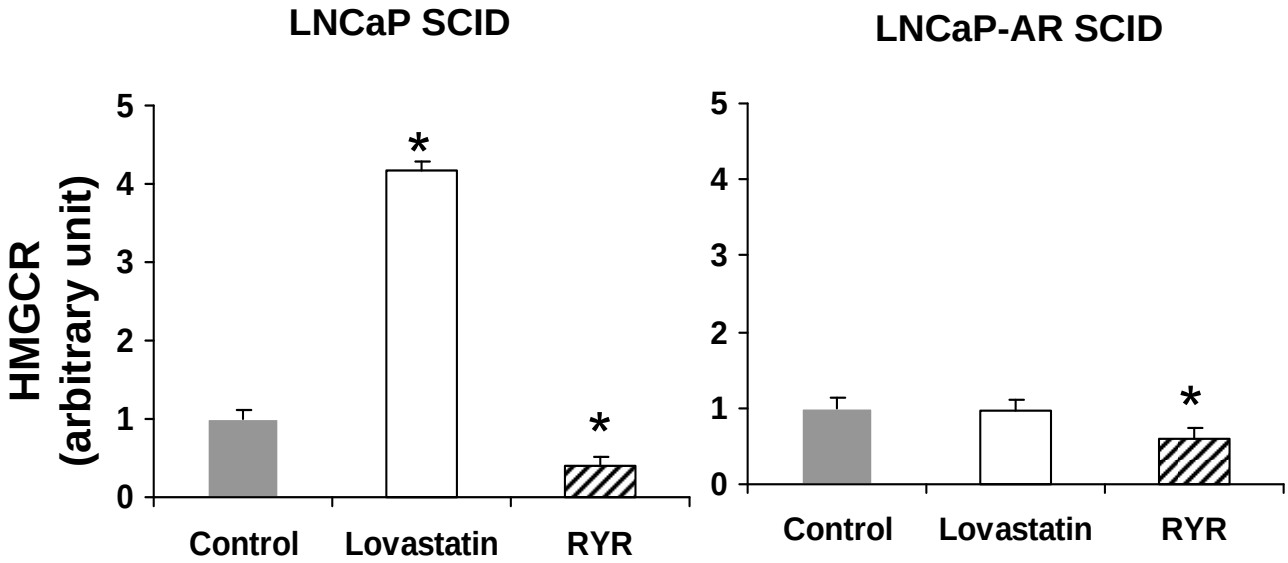

LNCaP SCID
LNCaP-AR SCID
HMGCR
(arbitrary unit)
5
4
3
2
1
0
*
*
*
Control
Lovastatin
RYR
Control
Lovastatin
RYR

Figure 6

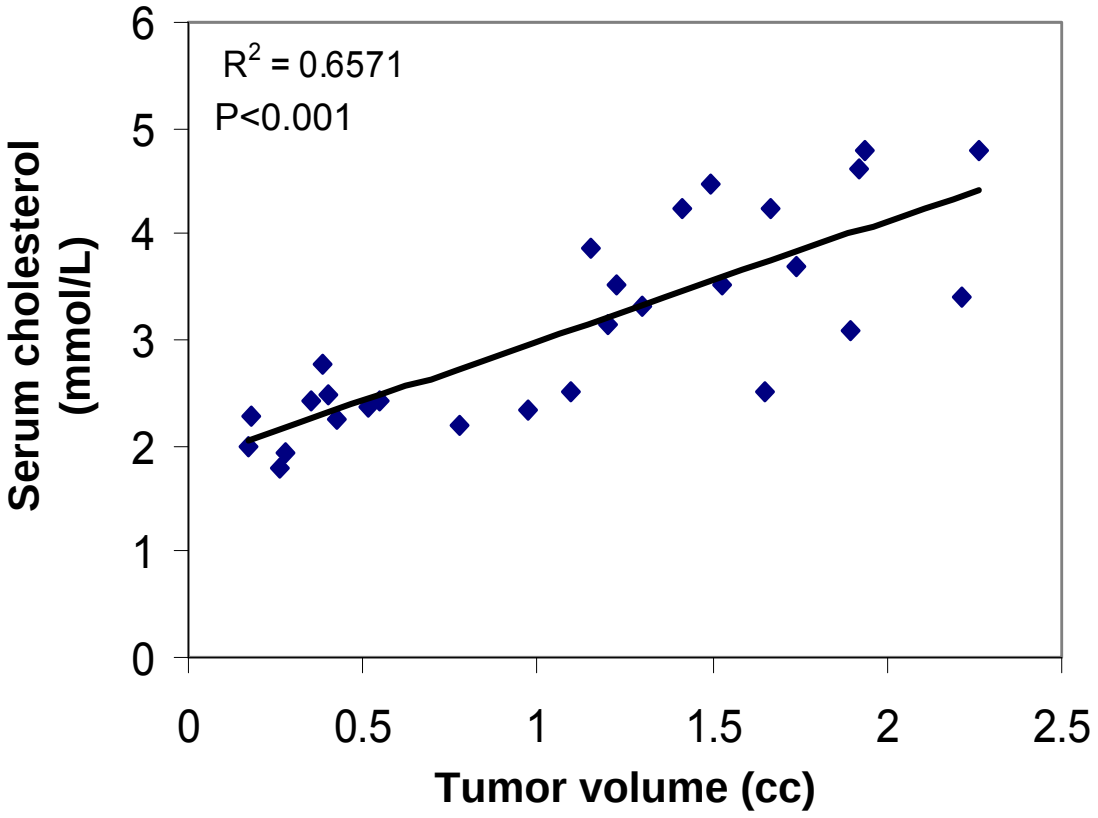

R^2 = 0.6571
P<0.001
Serum cholesterol (mmol/L)
Tumor volume (cc)
0
1
2
3
4
5
6
0
0.5
1
1.5
2
2.5

Figure 7

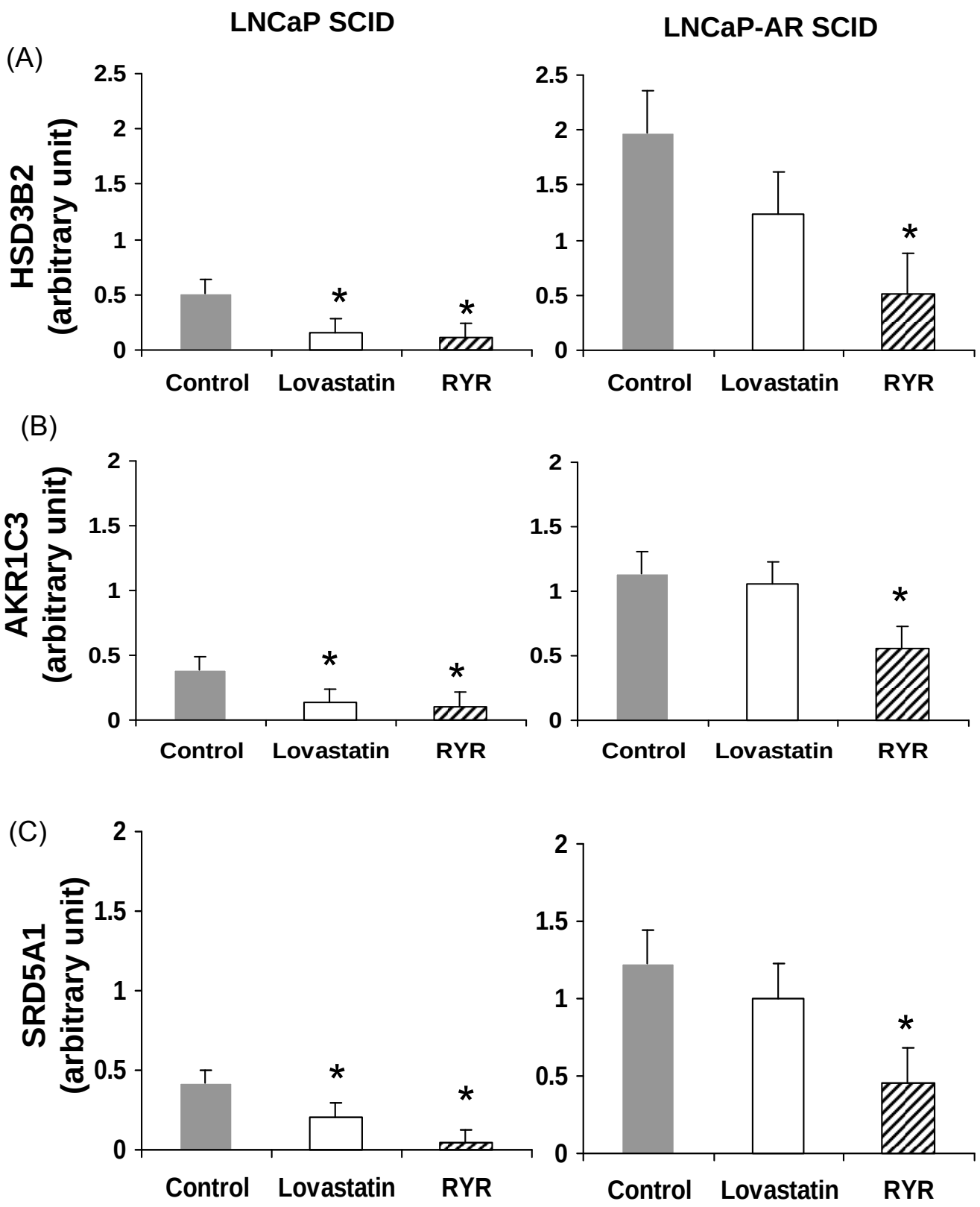

LNCaP SCID
LNCaP-AR SCID
(A)
HSD3B2 (arbitrary unit)
2.5
2
1.5
1
0.5
0
*
*
Control
Lovastatin
RYR
(B)
AKR1C3 (arbitrary unit)
(C)
SRD5A1 (arbitrary unit)

Figure 8

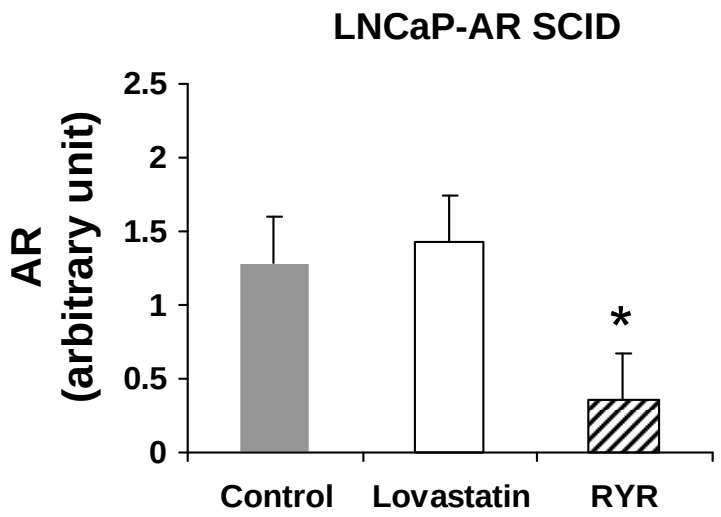

LNCaP-AR SCID
AR
(arbitrary unit)
2.5
2
1.5
1
0.5
0
*
Control
Lovastatin
RYR

Figure 9