Curcumin Inhibits Skin Squamous Cell Carcinoma Tumor Growth In Vivo

Article *in* Otolaryngology Head and Neck Surgery · March 2011
DOI: 10.1177/0194599811400711 · Source: PubMed

9 authors, including:

Lilantha Herman-Ferdinandez
Louisiana State University Health Sciences Center Shreveport
4 PUBLICATIONS **110** CITATIONS
SEE PROFILE

Tara Moore-Medlin
Louisiana State University Health Sciences Center Shreveport
33 PUBLICATIONS **389** CITATIONS
SEE PROFILE

John Clifford
Louisiana State University Health Sciences Center New Orleans
85 PUBLICATIONS **2,392** CITATIONS
SEE PROFILE

Fleurette Abreo
Louisiana State University Health Sciences Center Shreveport
105 PUBLICATIONS **2,060** CITATIONS
SEE PROFILE

Curcumin Inhibits Skin Squamous Cell Carcinoma Tumor Growth In Vivo

Jeffrey M. Phillips, Cheryl Clark, Lilantha Herman-Ferdinandez, Tara Moore-Medlin, Xiaohua Rong, Jennifer Roberts Gill, John L. Clifford, Fleurette Abreo and Cherie-Ann O. Nathan

Otolaryngology -- Head and Neck Surgery 2011 145: 58 originally published online 1 March 2011
DOI: 10.1177/0194599811400711

AMERICAN ACADEMY OF OTOLARYNGOLOGY–HEAD AND NECK SURGERY FOUNDATION

Otolaryngology–Head and Neck Surgery
145(1) 58–63

DOI: 10.1177/0194599811400711
http://otojournal.org
SAGE

Curcumin Inhibits Skin Squamous Cell Carcinoma Tumor Growth In Vivo

Jeffrey M. Phillips, MD[1], Cheryl Clark, PhD[1,2], Lilantha Herman-Ferdinandez, MD[1], Tara Moore-Medlin[1], Xiaohua Rong[1,2], Jennifer Roberts Gill[3], John L. Clifford, PhD[2,3], Fleurette Abreo, MD[2,3], and Cherie-Ann O. Nathan, MD[1,2]

No sponsorships or competing interests have been disclosed for this article.

Abstract

Objective. Squamous cell carcinoma (SCCa) has increased from 4% to 10% over 4 decades, stimulating interest in developing novel agents that slow sun-damaged skin progression. This is the first study evaluating the naturally occurring bioactive food compound curcumin on skin cancer xenografts. Low bioavailability of curcumin has slowed its transition to clinical trials. It is hypothesized that curcumin has growth-inhibitory effects through the MTOR pathway and chemopreventive potential in skin SCCa where local application could bypass bioavailability problems.

Study Design. A randomized experimental animal and laboratory study.

Setting. Louisiana State University Health Sciences Center, Shreveport, Louisiana.

Subjects and Methods. SCID mice were pretreated with 0, 5, or 15 mg of curcumin (n = 8 per group), 3 days prior to injecting 10^6 SRB12-p9 skin SCCa cells in each flank, and were gavaged daily thereafter. Tumor volumes were measured and tumors were harvested on day 24 when mice were sacrificed. Immunohistochemical analysis of pS6 expression (n = 3 per group) and tumor volumes in the 3 groups were compared using 1-way analysis of variance and pairwise comparisons were determined with the Tukey *t* test if overall comparisons were significant.

Results. Tumor volume increased 2.3 times faster in control mice compared with the group receiving 15 mg of curcumin (P = .0003). A significant difference in average tumor volumes was seen (P = .0012), especially with treatment of 15 mg of curcumin compared with control P = .0003). Curcumin inhibited S6 phosphorylation (P = .0027), suggesting inhibition of the MTOR pathway.

Conclusion. Curcumin appears to inhibit skin SCCa growth and blocks tumor progression by inhibiting pS6 even when gavage is used to deliver curcumin, indicating even more significant effects in future experiments with local application.

Keywords

squamous cell carcinoma, skin, curcumin, MTOR, pS6, chemoprevention

Received September 10, 2010; revised January 25, 2011; accepted January 26, 2011.

Cutaneous squamous cell carcinoma (SCCa) is one of the most common human malignancies and represents approximately 20% to 25% of nonmelanoma skin cancers.[1] Although exact incidence is difficult to determine, given that nonmelanoma skin cancer (NMSC) is not frequently reported to cancer registries, estimated incidence is between 1 and 2 million cases annually and has continued to increase from 4% to 10% over the past several decades.[2] Additionally, SCCa carries the highest mortality rate of all skin cancers in adults over age 85 years.[3] Approximately 70% of cases of SCCa of the skin are found in the head and neck.[2]

Development of SCCa is multifactorial and is attributed to both environmental and genetic risk factors. The cumulative effect of chronic exposure to UV radiation remains the most important etiologic factor in development of SCCa. Similarly, the incidence of cutaneous SCCa increases with advancing age and decreases with geographic distance from the equator.[2] Individuals may develop SCCa in chronic inflammatory skin lesions, scars, burns, or ulcers, and patients with systemic immunosuppression are at risk. Other

[1]Department of Otolaryngology–Head & Neck Surgery, Louisiana State University Health Sciences Center, Shreveport, Louisiana, USA
[2]Feist-Weiller Cancer Center, Louisiana State University Health Sciences Center, Shreveport, Louisiana, USA
[3]Louisiana State University Health Sciences Center–Shreveport, Shreveport, Louisiana, USA

This article was presented at the 2010 AAO-HNSF Annual Meeting & OTO EXPO; September 26-29, 2010; Boston, Massachusetts.

Corresponding Author:
Cherie-Ann O. Nathan, MD Department of Otolaryngology–Head & Neck Surgery, Louisiana State University Health Sciences Center, 1501 Kings Highway, Shreveport, LA 71130-3932, USA
Email: cnatha@lsuhsc.edu

known risk factors include chronic exposure to ionizing radiation, arsenic exposure, and history of nonmelanoma skin cancer. Patients with inherited disorders of the skin, including xeroderma pigmentosum and oculocutaneous albinism, are at increased risk for SCCa.[4] Treatment of SCCa has focused on local medical and surgical interventions, ranging from cryotherapy or 5-flurouracil (5-FU) topical treatments of precancerous actinic keratoses[5] to electrodessication, curettage, or imiquimod therapy for smaller, more superficial lesions.[6] However, surgical excision using Mohs micrographic surgery or formal excision with margins is the most widely performed and effective treatment modality used in practice.[7]

Although local excision of smaller cutaneous SCCa is performed frequently with acceptable outcomes, patients with multiple lesions or condemned skin, particularly in the head and neck, pose a more difficult challenge. The frequency of visits to the clinic or operating room to manage these lesions often leads to untoward disfigurement and cost to the patient. Indeed, because of its high prevalence and frequent need for multiple surgical interventions, cutaneous SCCa has become one of the most costly cancers in the United States.[8]

Given the increasing incidence and morbidity of cutaneous SCCa, research has shifted focus toward chemoprevention of cutaneous malignancies, and multiple agents have been investigated. Oral retinoids have been shown to inhibit SCCa tumor growth in vitro and may prevent SCCa in some patients at high risk for nonmelanoma skin cancer.[9] Regular use of nonsteroidal anti-inflammatory drugs, such as etodolac and celecoxib, have been shown to decrease the risk of actinic keratosis and SCCa formation in animal models; however, case-control studies in the human population have resulted in conflicting evidence.[10] Black tea and green tea are polyphenol substances that have been investigated for their ability to inhibit ultraviolet B and chemically induced carcinogenesis in skin, although human clinical trials are limited.[11] Numerous other naturally occurring chemopreventive agents have been studied, including β-carotenes, lycopene, genistein, selenium, myricetin, and ginger, in both oral and topical preparations, with mixed results.[12]

More recently, curcumin, a naturally occurring polyphenolic compound, has been investigated for its role as a chemopreventive agent in a variety of cancers. Curcumin is the active ingredient in turmeric, an orange-yellow spice that has been used in ancient medicine dating back to 600 BC, and has been shown to have activity as an anti-inflammatory, antibacterial, and antioxidant agent. It has been used in a variety of disease processes involving nearly every organ system, with benefits such as providing cardioprotective effects and promoting digestive health.[13] Curcumin has been studied as an anticarcinogenic agent in the inhibition of pancreatic, colon, liver, hematologic, and oral cavity cancer[13] and has shown to be safe and nontoxic to humans at biologically effective doses in phase 1 clinical trials.[14] Its molecular mechanism of action in inhibiting tumor initiation, growth, and protein synthesis has yet to be clearly defined; however, in vitro studies have indicated its effect in AKT/MTOR intracellular signaling, nuclear factor *k*B (NF-*k*B) activation, STAT phosphorylation, and numerous transcription factors and protein kinases that are upregulated in carcinogenesis.[15] Despite promising data in the laboratory setting, poor bioavailability of curcumin attributable to poor absorption in the gut and rapid metabolism and elimination from the body has led researchers to question its clinical efficacy as a potential anticarcinogenic agent.[16]

Curcumin has also been explored in the treatment of skin cancer and other skin diseases. Limited in vitro and in vivo mouse studies have shown that curcumin inhibits 12-O-tetradecanoylphorbol-13-acetate (TPA)–induced expression of proto-oncogene messenger RNAs, alters arachidonic acid metabolism, and downregulates formation of transcription factor AP-1 and NF-*k*B in skin tumorigenesis.[17-19] AKT activation in intracellular signaling has been cited as one of the most critical steps in mouse skin tumor initiation and progression.[20] However, no data exist on curcumin's effects on the AKT/MTOR pathway in skin cancer. Therefore, we wanted to determine whether curcumin has growth-inhibitory effects on cutaneous SCCa using an in vivo murine model. We hypothesized that oral application of curcumin would have an inhibitory effect on tumor growth and that these effects could possibly be mediated through the AKT/MTOR intracellular signaling mechanisms in SCCa tumorigenesis.

Methods

Curcumin

Curcumin paste was prepared by suspending Curcumin (C3) Complex (0, 5, or 15 mg) powder (Sabinsa Corp., East Windsor, New Jersey) in 100 μL of corn oil for oral gavage feeding. Doses administered were 5 mg/d and 15 mg/d.

Cell Lines and Xenografts

The human skin SCC cell line SRB12-p9 was derived by single-cell cloning from SRB12 cells (a gift from Dr Reuben Lotan, Department of Thoracic Head and Neck Medical Oncology, University of Texas M.D. Anderson Cancer Center) and cultured as described.[21] This cell line was chosen because of its sensitivity to curcumin as evidenced in cell culture studies.

Cutaneous Squamous Cell Carcinoma Xenograft In Vivo Study

Severe combined immunodeficiency (SCID) mice used in the study were housed in a barrier facility, given a normal diet ad libitum, and maintained as formally approved by Louisiana State University Health Sciences Center Institutional Animal Care and Use Committee in accordance with US National Institutes of Health guidelines. Mice were pretreated with either 0 mg of curcumin (corn oil) or 5-mg or 15-mg curcumin paste by oral gavage once daily for 3 days prior to SCCa xenograft injection (n = 8 per group). Mice were then shaved and injected subcutaneously with 1×10^6 SRB12-p9 cells suspended in sterile phosphate buffered saline (day 0). All mice continued daily gavage with either 0 mg, 5 mg, or 15 mg of curcumin paste, and tumors were measured daily using digital calipers. Tumor volume (mm^3)

was calculated using the following formula: 0.52 × length^2 × width. Body weight was measured daily, and mice were monitored for adverse effects from the experiment. Daily oral gavage and tumor volume measurement continued through day 24, at which time tumors were harvested.

Immunohistochemical Analysis of Molecular Markers in Skin Squamous Cell Carcinoma

Tumors harvested on day 24 were embedded in paraffin, sectioned, and stained with hematoxylin and eosin for confirmation of SCCa presence by our study pathologist (F.A.). Tumors (n = 3 per group) were then stained with Ki67, a marker of cell-cycling cellular proliferation. Slides were read and scored by the pathologist, who was blinded to the study. To examine curcumin's effects on cell proliferation, the number of proliferating cells was determined in tumor sections from control, 5-mg, and 15-mg curcumin treatment groups stained with Ki67 antibody. The intense brown–stained Ki67+ cells were counted at ×200 magnification from 3 random high-power fields per tumor section and averaged (n = 3 per group).

Western Blot Analysis of Tumors

Soluble proteins extracted from cell lines and tumor cells were analyzed by Western blot, as previously described.[22] Western blots were developed with a secondary anti-rabbit antibody conjugated to horseradish peroxidase. Proteins were detected using enhanced chemiluminescence (Amersham Pharmacia Biotech, Piscataway, New Jersey) and analyzed with ImageQuant TL7.0 (GE Healthcare) software (n = 3 per group). The following antibodies were used: rabbit polyclonal primary antibodies from Cell Signaling (Beverly, Masachusetts): S6 ribosomal protein (1:100), phospho-S6 ribosomal protein (serine 235/236; 1:100), AKT (1:250), AKT (1:100), phospho-AKT (Ser473; 1:250), and actin (1:3500).

Statistics

Tumor volumes and densitometry scans for Western blot analysis of AKT, pAKT, S6, and pS6 expression in each of the 3 groups were compared using 1-way analysis of variance. Pairwise comparisons were determined with Tukey *t* test if individual comparisons were significant between treatment groups.

Results

Curcumin Inhibits Squamous Cell Carcinoma Tumor Growth

After injection of SRB12-p9 SCCa cells into the flank of each mouse, tumor size became large enough by day 7 to be recorded by the digital calipers. Tumor volume for each group (n = 8 per group) was averaged and recorded. A significant difference in tumor growth rate was seen between mice treated with 15 mg of curcumin compared with control (**Figure 1A**). Tumor volume increased 2.3 times faster in control mice compared with the 15-mg curcumin group. Overall average tumor volume was significantly smaller in the curcumin-treated group compared with the control group (P = .0012), particularly in the mice treated with 15 mg of curcumin compared with the control group (P = .0003). There was a significant difference in average tumor volumes between control and treatment groups at 5 mg of curcumin for only the early time points (days 0-16, $F_{1,16}$ = 14.36, P = .0016) when tumors were of smaller volumes. When repeated at a higher dose, there was a significant difference in tumor volume between control and 15 mg of curcumin at early time points (days 0-16, $F_{1,16}$ = 12.64, P = .0032) and late time points (days 17-24, $F_{1,16}$ = 82.99, $P < .001$). No significant difference was noted between 5 mg and 15 mg of curcumin at the early time points (days 0-16, $F_{1,16}$ = 1.00, P = .33), although a significant difference in tumor volumes was noted between 5 mg and 15 mg of curcumin at the later time points (days 17-24, $F_{1,16}$ = 19.33, P = .006). Weights remained stable in both control and treated mice that survived through tumor harvesting on day 24, suggesting no toxicity from curcumin treatment. Upon tumor harvesting, control mice had markedly bulkier, irregular, and invasive flank tumors, whereas curcumin-treated mice had smaller and more well-confined tumors (**Figure 1B**).

Curcumin Inhibits S6 Phosphorylation

We evaluated MTOR pathway inhibition in skin SCCa. Total levels of AKT and pAKT were unaffected (P = .41) (**Figure 2**). Phosphorylated S6 is a well-accepted marker of MTOR activity in clinical trials, and hence we sought to determine whether pS6 was downregulated with curcumin. Total S6 levels were unaffected (P = .04), whereas phosphorylated S6 expression was significantly decreased in tumors from mice treated with 15 mg of curcumin compared with control mice (P = .0027) (**Figure 2**).

S6 phosphorylation correlates with an increase in proteins involved in cell cycle progression.[23] Because curcumin inhibited S6 phosphorylation, we wanted to determine curcumin's effects on cellular proliferation. We noted a dose-dependent decrease in cellular proliferation as evidenced by a marked decrease in Ki-67 expression in tumor specimens from mice treated with curcumin compared with control mice ($F_{2,21}$ = 120.40, $P < .001$, **Figure 3**).

Discussion

Despite aggressive attempts to educate the public about the need to avoid prolonged UV radiation exposure and wear sunblock and sun-protective clothing, the incidence of nonmelanoma skin cancer continues to rise.[2] Given the increasing morbidity and cost of these prevalent cancers, the National Cancer Institute sponsored a large series of clinical trials to explore potential chemopreventive agents against a variety of common malignancies. With regard to nonmelanoma skin cancer, the goal of chemoprevention is to inhibit formation and progression of cutaneous malignancies after exposure to UV radiation has already occurred.[12] Oral retinoid therapy has been shown to be an effective chemopreventive agent in patients at moderate to high risk for nonmelanoma skin cancer. However, continued effectiveness relies on chronic use of these agents, which has been associated with hepatoxicity, hyperlipidemia, acute pancreatitis,

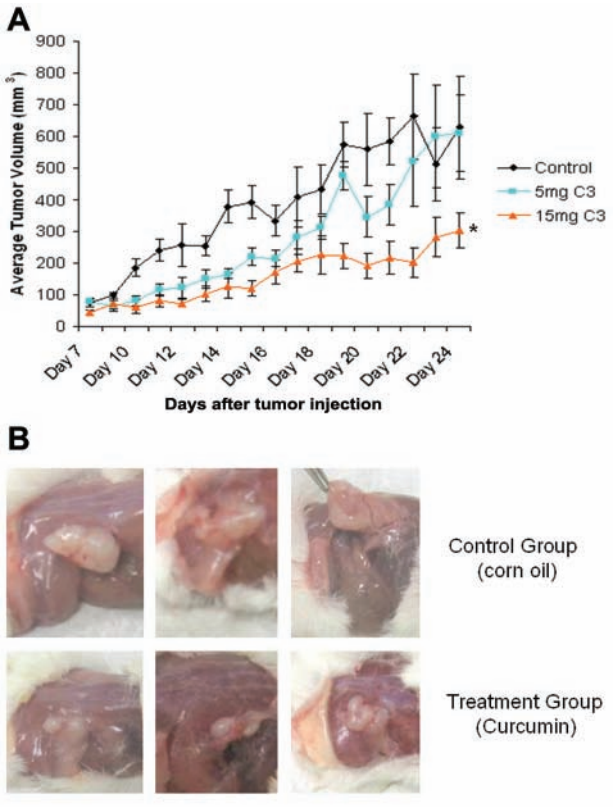

Figure 1. Curcumin activity in vivo. (A) Mice were pretreated with the indicated dose of curcumin for 3 days prior to injection with 1×10^6 SRB12-p9 skin tumor cells in each flank (day 0) and continued receiving daily curcumin treatment (8 mice per group, mean tumor volume ± SEM, *P = .0003 vs control group). (B) Representative tumors in control (– curcumin) and treatment (+ curcumin) groups.

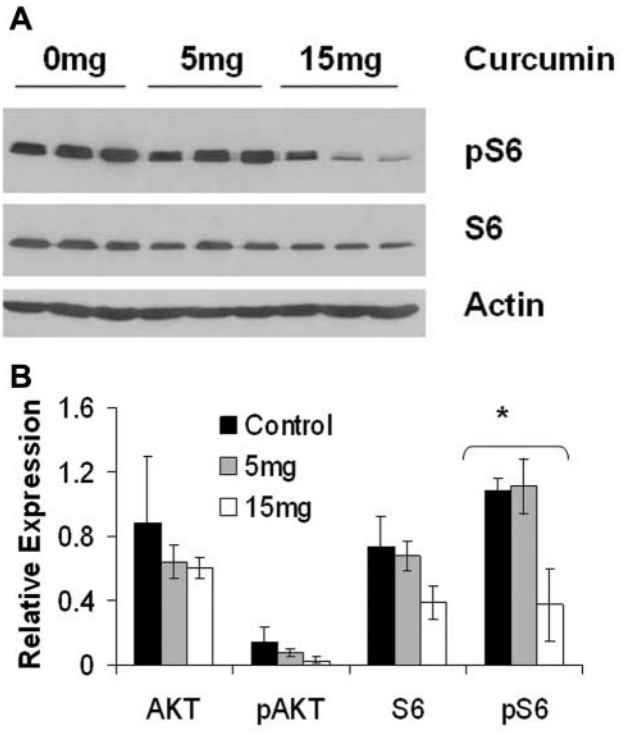

Figure 2. Curcumin inhibits pS6, a well-accepted downstream target of MTOR intracellular signaling. Whole-cell lysates were subjected to Western blot analysis with the indicated antibody and quantified by densitometry. Actin was used as a loading control. (A) Expression of pS6 was significantly decreased in the specimens treated with 15 mg of curcumin (*P = .0027). (B) Mean relative expression level of the indicated antibody at each dose (n = 3 per group) ± SD, *P = .0027).

hair loss, and teratogenicity.[12] More recently, research has focused on phytochemical compounds such as polyphenols, dietary flavonoids, and botanicals in chemoprevention of skin cancer. These naturally occurring substances have been shown to inhibit skin carcinogenesis through a variety of mechanisms, including antioxidant activity, inhibition of transcription factors in cellular signaling, and prevention of DNA damage and mutagenesis. Polyphenols are plant-derived, nonnutrient compounds with known antioxidant properties. Green tea and grape seed extracts have shown some promising results in mouse models, but no human clinical trials have been performed showing chemopreventive effects in skin cancer. Flavonoids, such as silymarin, genistein, and pomegranate, have shown efficacy in murine models as topical agents; however, data are limited.[12]

Curcumin, another polyphenolic compound, has been explored in the treatment of skin cancer and diseases such as psoriasis, scleroderma, and chronic wounds. Curcumin has been shown to lessen wound healing time, provide antioxidant protection, and improve collagen deposition.[13] Cheng et al[14] demonstrated in a phase 1 clinical trial that therapeutic doses of curcumin (up to 8000 mg/d) were safe and nontoxic to humans, and those investigators reviewed its chemopreventive effects in skin, liver, intestinal, and stomach carcinogenesis models. Subsequently, curcumin has been studied as a possible chemopreventive agent in melanoma and nonmelanoma skin cancer. Huang et al[17] first experimented with curcumin's inhibition of skin cancer induced by TPA in the mouse model and concluded that topically applied curcumin inhibited both TPA-induced tumor growth and arachidonic acid–induced inflammation in murine skin.[17,19] Limtrakul et al[18] further explored the anticarcinogenic effect of curcumin on DMBA-induced skin cancers in Swiss albino mice by the addition of curcumin to the diet and concluded that curcumin-treated mice developed fewer tumors and had decreased tumor volume compared with controls. These studies indicate that curcumin is an effective inhibitor of mouse skin carcinogenesis but highlight that curcumin's mechanism of action is unclear. Curcumin has been shown to inhibit NF-kB and induce apoptosis in mouse melanoma cells in vitro, suggesting curcumin's effect at an intracellular level.[24] In recent years, the AKT/MTOR pathway has been cited as essential in the initiation and progression of skin tumorigenesis; however, no data exist as to how curcumin might effect this pathway in skin carcinogenesis.[25]

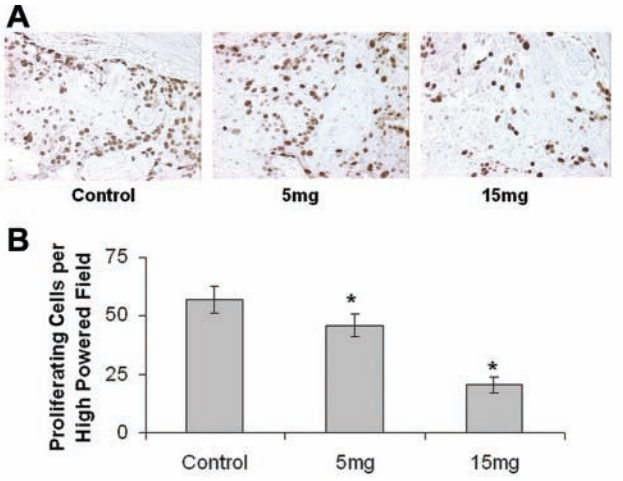

Figure 3. Curcumin inhibits cellular proliferation modulated by the MTOR pathway in vivo. Immunohistochemical staining with Ki67. (A) Representative brown nuclear staining of Ki67 expression in xenograft tumors from mice (n = 8 mice per group) treated with control (corn oil) and from mice treated with 5 mg or 15 mg of curcumin (shown at ×200 magnification). (B) Cell proliferation was quantified in tumor sections from the control group and the 5-mg and 15-mg curcumin treatment groups stained with Ki67 antibody. The intense brown-stained Ki67+ cells were counted at ×200 magnification from 3 random high-power fields per tumor and averaged (n = 3 per group). Mean Ki67 expression ± SD, *$P <$ .001 compared with control.

We sought to explore curcumin's effect on tumor growth and progression using cutaneous SCCa xenografts and hypothesized that any inhibitory effects on growth would be mediated by inhibition of biomarkers in the AKT/MTOR intracellular signaling pathway. Our in vivo data demonstrated sensitivity of SCCa xenografts to curcumin and significant inhibition of tumor growth. Although results appear dose-dependent, even treatment with 5 mg of curcumin demonstrated an inhibitory response, although not significant (**Figure 2**). Immunohistochemical staining for Ki-67 showed a significant decrease in expression, suggesting inhibition of tumor progression in mice treated with curcumin. The MTOR protein complex integrates signaling pathways that are often dysregulated in cancer,[26] making MTOR inhibition an attractive antitumor target. MTOR activity phosphorylates 4EBP1 through EIF4E[27] and regulates S6K1 that phosphorylates the 40S ribosomal protein S6. Our previous experience with curcumin in HNSCC cell lines[15] indicates that curcumin inhibits the MTOR signaling pathway. Inducible S6 phosphorylation is a known downstream biomarker linked to AKT/MTOR activation and cellular proliferation,[28] and the effects of curcumin on AKT have been shown to be dose-dependent.[15] Curcumin inhibited pS6 expression without inducing feedback activation of pAKT in our study, suggesting a possible effect of MTOR inhibition. This may be one of the mechanisms of curcumin's activity in skin SCCa.

Results from data published by Segrelles et al[25] and data from our study highlight the role of the AKT/MTOR pathway in skin carcinogenesis, however, no widely studied biomarker exists. As described above, NF-kB and transcription factor AP-1 have been investigated as potential targets for inhibition of cellular growth and protein synthesis. However, a number of intracellular signaling pathways exist that are specific to carcinogenic cells and may prove to be useful biomarkers in further studies in chemoprevention. The MEK/ERK pathway, induced by Ras-Raf protein kinase activation, has been studied in a variety of epidermal carcinogenesis models.[29] Receptor tyrosine kinases such as the epidermal growth factor receptor (EGFR) are often overexpressed and activated in SCCa. The EGFR is a key upstream activator of MAP kinase signaling that activates both the B-Raf/MEK/ERK pathway and Stat3 signaling pathways.[30] Retinoids are a potential chemopreventive agent for skin SCCa through inhibition of EGFR signaling at both early and late times during the chemical-induced skin SCCa.[30] However, side effects have precluded continuing clinical trials, and a search for safer compounds continues.

Although curcumin appears to be a safe and promising chemopreventive agent in skin carcinogenesis, many researchers have questioned its use as a therapeutic agent because of concerns over poor oral bioavailability. Curcumin is rapidly degraded in the gut and metabolized quickly, leading to low plasma levels and subsequently little substance distributed to target tissues.[18] Despite these concerns, our data showed significant tumor inhibition using oral curcumin, suggesting even more pronounced effects with controlled-release oral, topical, or nanoparticle curcumin preparations. Furthermore, we pretreated mice with daily oral curcumin administration and found a significant difference in tumor growth between the curcumin-treated group and control, even at early time points after placement of SCCa xenografts (**Figure 1**). This may be applicable to human studies in individuals with precancerous lesions and may represent a chemopreventive window of opportunity.

This the first study to demonstrate curcumin's inhibition of cutaneous SCCa in SCID mice. Inhibition of S6 phosphorylation indicates the possible effects of curcumin on the MTOR pathway, which has been shown to be activated in skin carcinogenesis. It is worth noting that although this study represents curcumin's inhibition of tumor growth of only a single very aggressive SCCa cell line in vivo, studies showing statistically significant inhibitory effects of curcumin on tumor growth using several other SCCa cell lines in vivo have been reported.[15] Overall, these data may promote future studies that explore local and controlled-release curcumin formulas in vivo. The search for other robust biomarkers in skin carcinogenesis continues, and further exploration of curcumin's effect on a variety of known intracellular signaling pathways is warranted.

Conclusion

As the incidence and associated morbidity of cutaneous SCCa increase, a shift in treatment paradigm toward chemoprevention has begun. Although a number of synthetic and naturally occurring biologic agents have been investigated, curcumin continues to demonstrate tumor inhibition both in vitro and in vivo while remaining safe at biologically efficacious doses.

The AKT/mTOR intracellular signaling mechanism appears to be central in skin tumorigenesis, and novel agents that target downstream moieties of this pathway may help prevent skin cancer formation and progression. This is the first study that demonstrates curcumin's inhibition of SCCa tumor growth in SCID mice resulting from inhibition of S6 phosphorylation. Although the bioavailability of curcumin via oral administration has been questioned, our study shows efficacy with this route, suggesting even more significant effects in future experiments with local application.

Author Contributions

Jeffrey M. Phillips, manuscript main author, experiment design, analysis, data acquisition; **Cheryl Clark**, research design, conception, article drafting and revision, data analysis; **Lilantha Herman-Ferdinandez**, data acquisition and analysis; **Tara Moore-Medlin**, data acquisition and analysis; **Xiaohua Rong**, data acquisition and analysis; **Jennifer Roberts Gill**, data acquisition and analysis; **John L. Clifford**, data analysis, research design, interpretation; **Fleurette Abreo**, data acquisition, analysis and interpretation; **Cherie Ann O. Nathan**, research design, conception, article drafting and revision, data analysis.

Disclosures

Competing interests: None.

Sponsorships: None.

Funding source: None.

References

1. Alam M, Ratner D. Cutaneous squamous-cell carcinoma. *N Engl J Med*. 2001;344:975-983.
2. Arora A, Attwood J. Common skin cancers and their precursors. *Surg Clin North Am*. 2009;89:703-712.
3. Weinstock M. Death from skin cancer among the elderly: epidemiological patterns. *Arch Dermatol*. 1997;133:1207-1209.
4. Parrish J. Immunosuppression, skin cancer, and ultraviolet A radiation. *N Engl J Med*. 2005;353:2712-2713.
5. Fenske N, Spencer J, Adam F. Actinic keratoses: past, present and future. *J Drugs Dermatol*. 2010;9:s45-s49.
6. Reschly M, Shenefelt P. Controversies in skin surgery: electrodessication and curettage versus excision for low-risk, small, well-differentiated squamous cell carcinomas. *J Drugs Dermatol*. 2010;9:773-776.
7. Minton T. Contemporary Mohs surgery applications. *Curr Opin Otolaryngol Head Neck Surg*. 2008;16:376-380.
8. Chen JG, Fleischer AB Jr, Smith ED, et al. Cost of nonmelanoma skin cancer treatment in the United States. *Dermatol Surg*. 2001;27:1035-1038.
9. Marquez C, Bair S, Smithberger E, et al. Systemic retinoids for chemoprevention of non-melanoma skin cancer in high-risk patients. *J Drugs Dermatol*. 2010;9:753-758.
10. Butler G, Neale R, Green A, et al. Nonsteroidal anti-inflammatory drugs and the risk of actinic keratoses and squamous cell cancers of the skin. *J Am Acad Dermatol*. 2005;53:966-972.
11. Katiyar S. Skin photoprotection by green tea: antioxidant and immunomodulatory effects. *Curr Drug Targets Immune Endocr Metabol Disord*. 2003;3:234-242.
12. Wright T, Spencer J, Flowers F. Chemoprevention of nonmelanoma skin cancer. *J Am Acad Dermatol*. 2006;54:933-946.
13. Pari L, Tewas D, Eckel J. Role of curcumin in health and disease. *Arch Physiol Biochem*. 2008;114:127-149.
14. Cheng A, Hsu C, Lin J, et al. Phase I clinical trial of curcumin, a chemopreventive agent, in patients with high-risk or pre-malignant lesions. *Anticancer Res*. 2001;21:2895-2900.
15. Clark C, McEachern M, Shah S, et al. Curcumin inhibits nicotine-induced activation of the AKT/MTOR pathway in HNSCC. *Cancer Prev Res*. 2010;3:1-10.
16. Shehzad A, Khan S, Shehzad O, et al. Curcumin therapeutic promises and bioavailability in colorectal cancer. *Drugs Today (Barc)*. 2010;46:523-532.
17. Huang M, Ma W, Yen P, et al. Inhibitory effects of topical application of low doses of curcumin on 12-O-tetradecanoylphorbol-13-acetate-induced tumor promotion and oxidized DNA bases in mouse epidermis. *Carcinogenesis*. 1997;18:83-88.
18. Limtrakul P, Lipigorngoson S, Namwong O, et al. Inhibitory effect of dietary curcumin on skin carcinogenesis in mice. *Cancer Lett*. 1997;116:197-203.
19. Conney A, Lysz T, Ferraro T, et al. Inhibitory effect of curcumin and some related dietary compounds on tumor promotion and arachidonic acid metabolism in mouse skin. *Adv Enzyme Regul*. 1991;31:385-396.
20. Segrelles C, Moral M, Lara M, et al. Molecular determinants of Akt-induced keratinocyte transformation. *Oncogene*. 2006;25:1174-1185.
21. Kleiner-Hancock H, Shi R, Remeika A, et al. Effects of ATRA combined with citrus and ginger-derived compounds in human SCC xenografts. *BMC Cancer*. 2010;10:394.
22. Nathan C, Amirghahari N, Abreo F, et al. Overexpressed eIF4E is functionally active in surgical margins of head and neck cancer patients via activation of the Akt/mammalian target of rapamycin pathway. *Clin Cancer Res*. 2004;10:5820-5827.
23. Peterson R, Schreiber S. Translation control: connecting mitogens and the ribosome. *Curr Biol*. 1998;8:R248-R250.
24. Marín Y, Wall B, Wang S, et al. Curcumin downregulates the constitutive activity of NF-kappaB and induces apoptosis in novel mouse melanoma cells. *Melanoma Res*. 2007;17:274-283.
25. Segrelles C, Ruiz S, Perez P, et al. Functional roles of Akt signaling in mouse skin tumorigenesis. *Oncogene*. 2002;21:53-64.
26. Molinolo A, Amornphimoltham P, Squarize C, et al. Dysregulated molecular networks in head and neck carcinogenesis. *Oral Oncol*. 2009;45:324-334.
27. Meric-Bernstam F, Gonzalez-Angulo A. Targeting the mTOR signaling network for cancer therapy. *J Clin Oncol*. 2009;27:2278-2287.
28. Thomas G. The S6 kinase signaling pathway in the control of development and growth. *Biol Res*. 2002;35:305-313.
29. Cooper J, Pajak T, Forastiere A, et al. Postoperative concurrent radiotherapy and chemotherapy for high-risk squamous-cell carcinoma of the head and neck. *N Engl J Med*. 2004;350:1937-1944.
30. Syed A, Cheepala S, Gill J, et al. All-trans retinoic acid suppresses Stat3 signaling during skin carcinogenesis. *Cancer Prev Res*. 2009;2:903-911.