The Challenge of Herbal Therapies for Prostate Cancer

□□ *Commentary on Singh et al., p. 7773*

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Abstract The paper by Singh et al. shows that silibinin inhibits prostate cancer growth in the TRAMP mouse model. Among its other properties, silibinin inhibits the progression of prostate cancer by decreasing angiogenesis and tumor invasion. Identification of the biological effects of herbal remedies such as silibinin is critical for their development as anticancer treatments.

There has long been a keen interest in herbal or alternative therapies for prostate cancer, particularly those that may not have the same side effects as androgen deprivation therapy. In this issue of *CCR*, Singh et al. (1) describe intriguing properties for how silibinin, a flavonolignan derived from seeds from the milk thistle plant, affects prostate cancer growth in an animal model. Perhaps the most interest in herbal therapy exists for patients progressing after primary surgery or radiation therapy, many of whom will exhibit rising prostate-specific antigen levels as their only manifestation of prostate cancer for several years before the detection of metastatic disease. In this setting, patients and clinicians are faced with the perplexing problem of watching prostate-specific antigen values increase in the absence of any other signs of disease progression, yet not knowing if any therapy for this stage is useful or prolongs life. Although androgen deprivation therapy is often prescribed for these patients, its value in this setting is not certain, and there has been an increasing appreciation of its short- and possible long-term morbidity (2, 3).

In the 1990s, an assortment of herbs from China, PC-SPES, was available primarily through internet sales. Although this product never went through the normal drug screening required by the Food and Drug Administration, it was used by thousands of patients with various stages of prostate cancer. Given its widespread use, our group and others tried to conduct clinical trials with PC-SPES (4, 5). Trials were halted and PC-SPES was removed from the market with the recognition that some lots were contaminated with minute quantities of warfarin or DES (4). Physicians gradually also came to realize that PC-SPES was a form of androgen deprivation therapy that had sufficient concentrations of phytoestrogens as to be thrombogeneic, require prophylatic low-dose warfarin therapy, and cause gynecomastia (4, 5). However, early results in castrate-resistant patients also suggested that PC-SPES was working by more than just a hormonal mechanism as it had a better response than DES in a two-arm trial (4).

The widespread use and possible success of PC-SPES led to a search for other "natural" or "herbal" remedies after it became unavailable. Today, as many as one-third of prostate cancer patients use alternative medications (6). In most cases, these therapies are not endorsed and often not even monitored by physicians, making it hard to discern their value.

Experiments by Singh et al. in the TRAMP prostate cancer tumor model show that silibinin treatment decreased proliferation and increased apoptosis in TRAMP tumors (1). In the current and in a similar recently published paper (7), their group showed that silibinin is effective in all stages of tumor progression, not only decreasing tumor formation when given before tumor establishment and decreasing metastatic potential but also significantly reducing the size of established palpable tumors and preventing premalignant cancers from becoming malignant. These properties make this a potentially interesting agent for patients with rapidly rising prostate-specific antigen values after surgery or radiation, possibly preventing or delaying the use of androgen deprivation therapy.

Given the robust effects of the agent in inhibiting different stages of prostate disease, Singh et al. wisely chose to focus on multiple key steps involved in tumor progression, including tumor invasion, angiogenesis, and metastasis (Fig. 1). A critical unifying factor for these steps in tumor progression is its invasive and angiogenic capacity. Silibinin treatment was associated with repression of snail-1 expression, and a corresponding increase in E-cadherin levels and decrease in vimentin levels. Increased expression of matrix metalloproteinases may also facilitate tumor invasion, and silibinin reduced levels of matrix metalloproteinase-2 and matrix metalloproteinase-3 in TRAMP tumors. Both in this and their recent *Cancer Research* paper (7) the authors show that vascular endothelial growth factor (VEGF) levels and VEGF receptor 2 (KDR, Flk-1) are decreased after silibinin treatment, which may explain part of its antiangiogeneic properties.

Although Singh et al. have shown several functional consequences of silibinin administration, it would be interesting to determine the underlying mechanism(s) by which proliferation, apoptosis, angiogenesis, and tumor progression could be so strongly affected. In an accompanying paper, they show that HIF-1 α levels are decreased in TRAMP tumors. A potential mechanism that would explain decreased expression of HIF-1α and VEGF is inhibition of the phosphatidylinositol-3-OH kinase/AKT/mammalian target of rapamycin pathway. Of interest also is the mechanism of decreased snail-1 expression, which in some

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Received 10/8/08; accepted 10/13/08.

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doi:10.1158/1078-0432.CCR-08-2316

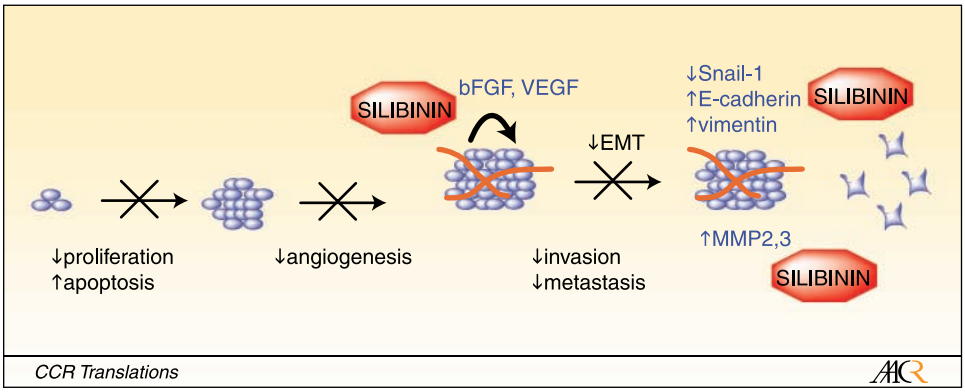

Fig. 1. Effects of silibinin on several prerequisite steps for tumor progression. Among these events are tumor cell proliferation and apoptosis both of which are blocked by silibinin. Events for which the authors show mechanistic data are indicated by red "stops signs." Angiogenesis is inhibited possibly by silibinin's reduction of basic fibroblast growth factor (bFGF) and VEGF secretion. Invasion and metastasis of tumors are also inhibited by a reduction in EMT, possibly as a result of decreased matrix metalloproteinase (MMP) production and increased levels of E-cadherin.

experimental systems is regulated by the expression of a homeobox gene, *HOXA10* (8). The effect of silibinin on some of these molecular pathways should be investigated.

One caveat of the TRAMP model is that it is not an appropriate model to study an agent if it has antiandrogenic properties. TRAMP mice develop prostate tumors because the tissue-specific promoter, probasin, drives expression of the SV40 early genes in prostatic epithelium. Probasin is androgen regulated, so that androgen deprivation therapy in this setting reduces transgene expression and may lead to tumor regression by this mechanism alone. It is encouraging that silibinin has shown antitumor activity in other nonhormonal tumor models such as bladder and kidney cancer (9, 10). Nonetheless it may be important to ensure that silibinin does not affect the expression of an androgen-driven promoter.

An advantage of studying the antitumor effects of silibinin in animal models is the identification of possible biomarkers that might be investigated in clinical trials. If possible, microvessel density, E-cadherin, and vimentin expression in tumors from silibinin-treated patients should be assessed. However, it is difficult to obtain tumor tissue from patients on trials, especially paired samples, and these markers can be challenging to assess by immunohistochemistry. If silibinin proves to be extremely safe in ongoing clinical trials, it might be studied in the neoadjuvant setting in which at least adequate prostate samples could be obtained from patients after prostatectomy.

Singh et al. show that silibinin treatment reduces both VEGF and levels basic fibroblast growth factor in blood samples from treated animals. Biomarkers from serum or plasma, can, of course, be obtained repeatedly during the course of a trial, unlike tumor tissue. Their findings suggest that the tumors in TRAMP mice produce sufficient levels of these cytokines to be detectable above background levels. Although some studies have shown that VEGF and some of matrix metalloproteinases might serve as biomarkers of disease progression in prostate cancer (11, 12), it is likely that in many clinical settings insufficient levels of these cytokines are produced from tumors, and therefore, only dramatic changes during treatment would be detectable above background levels (13). Nonetheless, this work suggests that at a minimum both serum and plasma samples should be obtained at baseline, during the study, and especially at progression in clinical trials of silibilin.

It is fascinating that an agent derived from seeds from what is a fairly common weed can have such profound biological effects. There are multiple advertisements on the internet that both sell and promote silibinin, primarily as an agent used for liver disease and as an antioxidant. But it is often sold as a component of other herbal remedies and there is no way to be certain of the agent's purity. The challenge of herbal or alternative therapy is to perform the type of studies by Singh et al. that systematically identify the biological and biochemical consequences of an herbal remedy in a relevant tumor model. Furthermore, studies that identify the effect of an agent on specific biochemical pathways would greatly advance the field. Although it is likely that combinations of different herbs or compounds could have an additive or even synergistic effect, unless we approach the investigation of these agents in a stepwise manner, this area will be consistently dogged by anecdotal reports with little science to build upon, making our patients continual prey to unsubstantiated internet advertisements.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Singh RP, Raina K, Sharma G, Agarwal R. Silibinin inhibits established prostate tumor growth, progression, invasion and metastasis, and suppresses tumor angiogenesis and epithelial-mesenchymal transition in transgenic adenocarcinoma of the mouse prostate model mice. Clin Cancer Res 2008;7773–80.
2. Yannucci J, Manola J, Garnick MB, Bhat G, Bubley GJ. The effect of androgen deprivation therapy on fasting serum lipid and glucose parameters. J Urol 2006;176:520–5.
3. Smith MR. Androgen deprivation therapy and risk for diabetes and cardiovascular disease in prostate cancer survivors. Curr Urol Rep 2008;9:197–202.
4. Oh WK, Kantoff PW, Weinberg V, et al. Prospective, multicenter, randomized phase II trial of the herbal supplement, PC-SPES, and diethylstilbestrol in patients with androgen-independent prostate cancer. J Clin Oncol 2004;22:3705–12.
5. Small EJ, Frohlich MW, Bok R, et al. Prospective trial of the herbal supplement PC-SPES in patients with progressive prostate cancer. J Clin Oncol. 2000;18:3595–603.
6. Clement J, Bubley G. Prostasol and venous thromboembolism. Urology 2008;72:664–6.
7. Raina K, Rajamanickam S, Singh RP, Deep G, Chittezhath M, Agarwal R. Stage-specific inhibitory effects and associated mechanisms of silibinin on tumor progression and metastasis in transgenic adenocarcinoma of the mouse prostate model. Cancer Res 2008;68:6822–30.
8. Yoshida H, Broaddus R, Cheng W, Xie S, Naora H. Deregulation of the HOXA10 homeobox gene in endometrial carcinoma: role in epithelial-mesenchymal transition. Cancer Res 2006;66:889–97.
9. Li L, Gao Y, Zhang L, Zeng J, He D, Sun Y. Silibinin inhibits cell growth and induces apoptosis by caspase activation, down-regulating survivin and blocking EGFR-ERK activation in renal cell carcinoma. Cancer Lett 2008.
10. Singh RP, Tyagi A, Sharma G, Mohan S, Agarwal R. Oral silibinin inhibits *in vivo* human bladder tumor xenograft growth involving down-regulation of survivin. Clin Cancer Res 2008;14:300–8.
11. Shariat SF, Karam JA, Walz J, et al. Improved prediction of disease relapse after radical prostatectomy through a panel of preoperative blood-based biomarkers. Clin Cancer Res 2008;14:3785–91.
12. De Cicco C, Ravasi L, Zorzino L, et al. Circulating levels of VCAM and MMP-2 may help identify patients with more aggressive prostate cancer. Curr Cancer Drug Targets 2008;8:199–206.
13. George DJ, Regan MM, Oh WK, et al. Radical prostatectomy lowers plasma vascular endothelial growth factor levels in patients with prostate cancer. Urology 2004;63:327–32.