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Promise and Potential of Silibinin in Colorectal Cancer Management: what patterns can be seen?

Komal Raina[1] and **Rajesh Agarwal**[1,2]

[1]Department of Pharmaceutical Sciences, Skaggs School of Pharmacy, University of Colorado Anschutz Medical Campus, Aurora, Colorado

[2]University of Colorado Cancer Center, University of Colorado Anschutz Medical Campus, Aurora, Colorado

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EDITORIAL

Colorectal cancer (CRC) is the third leading cause of cancer-related deaths in the US (both genders combined); statistical estimates for 2013 indicate ~142,820 new CRC cases and 50,830 associated deaths [1]. About 75% of sporadic CRC develop from adenomatous polyps/benign adenomas in the colon; while the rest 25% of the cases are due to inherited syndromes such as familial adenomatous polyposis. Furthermore, the risk of CRC is known to increase with other medical conditions, such as inflammatory bowel disease, obesity and type 2 diabetes [2]. In parallel, the global incidence of CRC has been rising over the past two decades, which has been attributed to the rise in obesity, frequency of associated diabetes, together driven by an increase in the intake of high calorie processed foods and lack of exercise [2]. However, overall, colon screening initiatives, prevention strategies, biomarker and genomic analysis, personalized interventions and chemotherapy have also caused a major impact on the management of CRC [1]. While removal of adenomatous polyps during screening helps prevent CRC, only part of the cases are diagnosed at this stage, mostly due to under use of screening. The undetected cancer spreads to various organs and the 5-year survival drops to 11% with distant metastasis [1].

"*Aegrescit medendo*", Latin for, "*the remedy is worse than the disease*" echoes the state of most of the CRC patients. For patients with localised CRC disease, colon resection is the treatment of choice [1]. The surgery often involves removal of the affected colon segment with its mesentery, vascular structures and the associated lymph nodes [1]. Some patients may still require adjuvant chemotherapy or adjuvant radiotherapy with or without concurrent chemotherapy depending on the stage of their malignancy [1]. Furthermore, biological therapies are also being incorporated as part of the treatment in advanced settings [1]. However, in spite of these treatment advances, CRC patients with advanced and metastatic stages of the disease still succumb to death. Also, nearly 50% of CRC patients develop recurrent disease even after aggressive surgical resection and chemotherapy [1, 3]. At the

Requests for reprints: Rajesh Agarwal, Rajesh.Agarwal@UCDenver.edu Department of Pharmaceutical Sciences, Skaggs School of Pharmacy and Pharmaceutical Sciences, University of Colorado Anschutz Medical Campus, 12850 E. Montview Blvd, C238, Room V20-2117, Aurora, CO 80045. Phone: (303) 724-4055, Fax: (303) 724-7266.

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same time, we cannot overlook the fact that the most effective anti-cancer drugs face limited clinical applications due to their high toxicity and increased chemoresistance [1].

Thus, combination therapy/chemoprevention is gaining increased attention as an effective alternative to increase therapeutic efficacy and to minimize the systemic toxicity of the chemotherapeutic agents. Accordingly, the interventions which reduce incidence of adenomatous polyps and/or prevent their progression to CRC are being developed [2, 4]. In this regard, several studies have reported the chemopreventive potential of a wide range of agents against CRC [4]. Non-steroidal anti-inflammatory drugs are the drugs of choice to prevent CRC [5]; however, there are several side effects associated with their long-term use suggesting that more studies are needed to identify non-toxic agents that can be used to prevent/intervene CRC [5].

Taking cue from the Latin phrase "*Medicus curat, natura sanat*" which means "doctor cures, nature saves", various efforts are being made world-wide to identify non-toxic, dietary/non-dietary agents sourced from 'Mother Nature' that have cancer chemopreventive potential against CRC [4]. In this regard, studies conducted mostly by Agarwal and colleagues, and others have established strong anti-cancer and cancer chemopreventive efficacy of silibinin, (a flavonolignan from milk thistle, *Silybum marianum*), in cell culture and animal models of various malignancies including CRC [4, 6]. Selection of silibinin is based on the facts that it is a widely consumed dietary supplement for hepatotoxicity around the world and has a long history of human use and is considered exceptionally safe [4, 6]. Regarding CRC, silibinin has shown strong preventive and therapeutic efficacy in different pre-clinical models through various mechanisms [4, 6–16]. Velmurugan *et al*., have reported that silibinin prevents azoxymethane (AOM)-induced formation of pre-neoplastic lesions, aberrant crypt foci, in Fisher 344 rats, in terms of a strong reduction in crypt multiplicity in both pre- & post-initiation and post-initiation treatments [16]. A study employing long-term silibinin feeding, by Ravichandran *et al*., also showed prevention of AOM-induced colon tumorigenesis in A/J mouse model [14]. In other studies by Rajamanickam *et al*., silibinin prevented spontaneous intestinal tumorigenesis in *APC*$^{min/+}$ mice as observed by a significant decrease in both number and size of intestinal including colon polyps [12, 13]. This effect of silibinin was associated with a decrease in proliferation and an increase in apoptotic indices in polyps. Importantly, the anti-proliferative and pro-apoptotic effect of silibinin were specific and limited to polyps, and not observed in normal crypt-villus regions of the intestine [12, 13].

Studies have also shown strong preventive and/or therapeutic efficacy of silibinin against CRC xenografts (HT29, LoVo and SW480) in nude mice [4, 8, 9, 15]. Mechanistic investigations in tissue samples from various animal studies revealed that the anti-CRC effects of silibinin were mainly due to the inhibition of Wnt/β-catenin pathway, which is known to play an essential role in the development and progression of CRC [8, 12]. These results were also supported by other detailed cell culture studies, where silibinin inhibited β-catenin dependent transcriptional activity of Tcf-4 in CRC cells, together with a decrease in the expression of its transcriptional targets, namely cyclin D1 and c-myc [8]. Studies have also shown strong silibinin effect on growth of various human CRC xenografts and cell lines *via* inhibition of proliferation, apoptosis/autophagy induction and modulation in PI3K/Akt/mTOR-dependent signaling [7–9, 11].

Furthermore, the inflammatory milieu of the colon is an important growth regulator for CRC [17]. The inflammatory signals such as cytokines and chemokines arising in the tumor niche, due to the presence of inflammatory cells, also influence the progression of CRC [17]. With regard to the anti-inflammatory effect of silibinin in CRC, studies by Raina *et al*. have shown that silibinin strongly inhibits TNFα-induced NF-κB activation in human CRC cells

[10]. In this regard, growth inhibitory effects of silibinin in xenograft, *APC*$^{min/+}$, and AOM-induced CRC models were also associated with its anti-inflammatory mechanisms involving decreased expression of COX2 and iNOS, as well as inhibition of NF-κB transcriptional activity [9, 10, 12–14]. Together, these results highlight the strong potential of silibinin in inhibiting the initiation and progression of CRC in various murine models.

From a broader view point, the limitation of these studies was that it did not discuss the efficacy of the silibinin treatment on colon cancer stem cells (CSC); this is important, as recent reports indicate towards CSC being the main cause for initiation, promotion and progression of most of the epithelial cancers, including CRC [3]. As an old Latin proverb cautions, *"Dulcior illa sapit caro, quae magis ossibus haeret"*, which means "*one must attack the root cause, don't try to cure the symptoms-cure the disease*", thus, discovery and development of the agents, especially chemopreventive agents, which target CSC – a well-recognized root cause of CRC, are urgently needed. This also has important implications because CSC are inherently resistant to chemo- and radio-therapies and are the major cause for failure of most of the current therapies [3, 18]. Targeting CSC by non-toxic approach might provide opportunities to intervene at the earliest and also at a late stage in cancer therapy, as it would eradicate CSC pool, which otherwise escapes the therapeutic insult resulting in cancer relapse [3, 18]. In this regard, Raina *et al*. recently reported that silibinin targets CSC 'self renewal' and 'differentiation' capabilities in CRC cell lines [19], thereby, confirming that silibinin possesses strong efficacy against CRC initiation, promotion and recurrence; these results, however, need to be further validated in *in vivo* scenario.

Furthermore, a metabolomics study utilizing quantitative high-resolution nuclear magnetic resonance spectroscopy (^{1}H-, ^{13}C- and ^{31}P-NMR), was carried out by Raina *et al*., to assess the metabolic profile and energy state of the silibinin-treated CRC cells [11]. Results show that silibinin harbors a deadly 'double-edged sword' against CRC cells in terms of inducing cell death, where first it initiates an apoptotic response triggered by induction of oxidative stress and then imposes energy restrictions within the cells, thereby resulting in induction of autophagic death [11]. Due to sustained interference in essential cellular processes such as mitochondrial metabolism, phospholipid and protein synthesis, the cellular damage to CRC cells by silibinin was severe and irreparable [11]. These results suggest that small molecules, like silibinin, thus, have the potential to be the 'golden bullets' as they have the potential to initiate different types of programmed cell-death [11]. Together, these findings advocate the use of silibinin as 'adjunct therapy' where current anti-cancer modalities fail to augment the anti-cancer effect due to a defective apoptotic machinery or resistance of CRC cells to the specific death mechanism induced by that treatment. Importantly, in their completed pilot study with silibinin in CRC patients, Hoh *et al*. have shown high bioavailability of silibinin in colonic tissue of CRC patients [20]. Given that silibinin consumption is safe, the ongoing research on the usefulness of silibinin has strong translational preventive and therapeutic implications in the management of CRC in humans.

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References

1. National Cancer Institute. PDQ® Genetics of Colorectal Cancer. Bethesda, MD: National Cancer Institute; Date last modified <02/28/2013>. Available at: http://cancer.gov/cancertopics/pdq/genetics/colorectal/HealthProfessional
2. Center MM, Jemal A, Ward E. International trends in colorectal cancer incidence rates. Cancer epidemiology, biomarkers & prevention. 2009; 18(6):1688–1694.

3. Huang EH, Wicha MS. Colon cancer stem cells: implications for prevention and therapy. Trends Mol Med. 2008; 14(11):503–509.
4. Rajamanickam S, Agarwal R. Natural products and colon cancer: current status and future prospects. Drug Dev Res. 2008; 69(7):460–471.
5. Elwood PC, Gallagher AM, Duthie GG, Mur LA, Morgan G. Aspirin, salicylates, and cancer. Lancet. 2009; 373(9671):1301–1309. [PubMed: 19328542]
6. Ramasamy K, Agarwal R. Multitargeted therapy of cancer by silymarin. Cancer letters. 2008; 269(2):352–362. [PubMed: 18472213]
7. Agarwal C, Singh RP, Dhanalakshmi S, et al. Silibinin upregulates the expression of cyclin-dependent kinase inhibitors and causes cell cycle arrest and apoptosis in human colon carcinoma HT-29 cells. Oncogene. 2003; 22(51):8271–8282.
8. Kaur M, Velmurugan B, Tyagi A, Agarwal C, Singh RP, Agarwal R. Silibinin suppresses growth of human colorectal carcinoma SW480 cells in culture and xenograft through down-regulation of beta-catenin-dependent signaling. Neoplasia. 2010; 12(5):415–424. [PubMed: 20454513]
9. Kaur M, Velmurugan B, Tyagi A, et al. Silibinin suppresses growth and induces apoptotic death of human colorectal carcinoma LoVo cells in culture and tumor xenograft. Mol Cancer Ther. 2009; 8(8):2366–2374.
10. Raina K, Agarwal C, Agarwal R. Effect of silibinin in human colorectal cancer cells: Targeting the activation of NF-kappaB signaling. Mol Carcinog. 2013; 52(3):195–206. [PubMed: 22086675]
11. Raina K, Agarwal C, Wadhwa R, Serkova NJ, Agarwal R. Energy deprivation by silibinin in colorectal cancer cells: A double-edged sword targeting both apoptotic and autophagic machineries. Autophagy. 2013; 9(5)
12. Rajamanickam S, Kaur M, Velmurugan B, Singh RP, Agarwal R. Silibinin suppresses spontaneous tumorigenesis in APC min/+ mouse model by modulating beta-catenin pathway. Pharm Res. 2009; 26(12):2558–2567. [PubMed: 19779968]
13. Rajamanickam S, Velmurugan B, Kaur M, Singh RP, Agarwal R. Chemoprevention of Intestinal Tumorigenesis in APCmin/+ Mice by Silibinin. Cancer Res. 2010; 70(6):2368–2378. [PubMed: 20215518]
14. Ravichandran K, Velmurugan B, Gu M, Singh RP, Agarwal R. Inhibitory effect of silibinin against azoxymethane-induced colon tumorigenesis in A/J mice. Clin Cancer Res. 2010; 16(18):4595–4606. [PubMed: 20823143]
15. Velmurugan B, Gangar SC, Kaur M, Tyagi A, Deep G, Agarwal R. Silibinin exerts sustained growth suppressive effect against human colon carcinoma SW480 xenograft by targeting multiple signaling molecules. Pharm Res. 2010; 27(10):2085–2097.
16. Velmurugan B, Singh RP, Tyagi A, Agarwal R. Inhibition of azoxymethane-induced colonic aberrant crypt foci formation by silibinin in male Fisher 344 rats. Cancer Prev Res (Phila). 2008; 1(5):376–384. [PubMed: 19138982]
17. Mantovani A. Molecular pathways linking inflammation and cancer. Curr Mol Med. 2010; 10(4): 369–373. [PubMed: 20455855]
18. Sanchez-Garcia I, Vicente-Duenas C, Cobaleda C. The theoretical basis of cancer-stem-cell-based therapeutics of cancer: can it be put into practice? Bioessays. 2007; 29(12):1269–1280.
19. Raina, K.; Kumar, S.; Tyagi, A.; Agarwal, C.; Agarwal, R. Silibinin causes strong efficacy towards colon cancer stem cells by modulating IL-4 caused CD44 expression [abstract]. Cancer Res; Proceedings of the 103rd Annual Meeting of the American Association for Cancer Research; 2012 Mar 31-Apr 4; Chicago, IL. Philadelphia (PA): AACR; 2012. Abstract nr 16371538-7445.AM2012-1637, 2012
20. Hoh C, Boocock D, Marczylo T, et al. Pilot study of oral silibinin, a putative chemopreventive agent, in colorectal cancer patients: silibinin levels in plasma, colorectum, and liver and their pharmacodynamic consequences. Clin Cancer Res. 2006; 12(9):2944–2950. [PubMed: 16675592]