Review Article

Inhibition of colon cancer cell growth by dietary components: role of the insulin-like growth factor (IGF) system

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Large bowel cancer is one of the leading causes of deaths from cancer in Western countries, and the incidence of colorectal cancer is increasing with the steady increase in life expectancy. Modification of diet and lifestyle provide measures of reducing the risk of developing colon cancer. Evidence suggests that the components of the insulin-like growth factor (IGF) system may be appropriate targets for cancer prevention and therapy. A positive correlation was found between dietary and lifestyle, plasma IGF-I, and colon cancer incidence rates. Diet, nutrition, and other lifestyle features affect the expression and production of IGF-1 and other members of the IGF family. The purpose of this review is to examine current evidence obtained from our recent studies and others that investigated the role of dietary components in the regulation of the IGF system and colon cancer cell growth.

Key Words: Conjugated linoleic acid, vitamin D, retinoic acid, n–3 fatty acids, IGF-I receptor, IGFBPs

INTRODUCTION

Colorectal cancer is one of the most common malignancies in the Western world, and the incidence of this disease is increasing worldwide. Surgical removal of the primary tumor combined with adjuvant chemotherapy for a subset of patients is the standard treatment for colon cancer. Unfortunately, despite intense efforts, in advanced cases of the disease, treatment has had limited success. Since cancer usually develops over a 10- to 20-year period and conventional treatments such as surgical resection, radiation therapy, and chemotherapy are still unsatisfactory, prevention of this disease or at least stopping it at its inception is important. Diet and lifestyle modifications offer means of reducing the risk of developing colon cancer. Epidemiologic studies combined with animal models and *in vitro* experiments indicate that natural components of the diet may serve as chemopreventive agents that suppress the growth and dissemination of neoplastic colon cells.

The dysregulation of the insulin-like growth factor (IGF) system should be considered an important cancer risk factor, and thus a potential target for new antineoplastic therapies and/or preventive strategies in high-risk groups.[1] The IGF system may play an important role in the proliferation of colon cancer.[2] The potential for dietary intervention to alter the IGF system thereby decreasing cancer risk, is supported by several lines of evidence.[3] In this review we focus on our recent research results from studies of the effect of various dietary components on colon cancer growth and the IGF system family members.

IGF system and colon cancer

The IGF system comprises the peptide growth factors IGF-I and -II, type I and II IGF receptors, IGF-binding proteins (IGFBPs) and their corresponding proteases.[4] IGF-I and -II play important roles in proliferation, differentiation and transformation in a wide variety of cell types. The actions of IGFs are mediated through the IGF-I receptor (IGF-IR). Ligand binding to IGF-IR induces receptor autophosphorylation in the intracellular domain of the β-subunit and results in activation of the intrinsic tyrosine kinase of the IGF-IR. Upon tyrosine phosphorylation of the IGF-IR, multiple intracellular substrates are recruited to "docking sites" formed by the phosphotyrosine. These include the insulin-receptor substrate (IRS) family of proteins and the Shc family of adaptor proteins. Signaling pathways known to be activated by IGF-IR activation include pathways involved in activation of the extracellular signal-regulated kinase (ERK) subfamily of mitogen-activated protein kinases (MAPKs) and a pathway involved in the activation of phosphatidylinositol 3-kinase (PI3K).[5]

Unlike insulin, the IGFs are bound to IGFBPs in the circulation and in extracellular fluids.[4] Six IGFBPs with high affinity to IGFs have been identified, and at least nine IGFBP-related proteins with low affinity have also been described.[6] Under certain conditions the IGFBPs inhibit IGF stimulation of the IGF-I receptor, whereas under other conditions, they may increase the IGF-stimulated IGF-I

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receptor activation. In addition, IGFBPs may have IGF-independent actions.[7]

Several elements of the IGF axis exhibit altered expression and thereby may play an important role in the development of colon cancer.[2] Positive associations were found between plasma IGF-I and colorectal cancer risk in North American,[8] Northern Sweden,[9] and multiethnic cohorts.[10] The single most overexpressed gene in colorectal cancer relative to normal colonic epithelial cells is IGF-II and elevated circulating levels of IGF-II are associated with the development of colorectal cancer.[11,12] IGFBP-2 levels are also increased in sera and tumors from patients with colonic neoplasia.[13] Utilizing liver specific IGF-I-deficient mice in which serum IGF-I levels are 25% of that in control mice, Wu *et al*.[14] have shown that circulating IGF-I levels regulate growth and metastasis of orthotopically transplanted colon tumors.

The human colon adenocarcinoma cell lines, Caco-2, HCT116, COLO 205, COLO 320 DM, LoVo, DLD-1, SW480 and HT29, secrete IGF-II and several IGFBPs,[15-19] and IGF-II acts as an autocrine growth stimulator of these cells.[20-22] IGF-II up-regulates COX-2 expression in Caco-2 cells; this up-regulation is mediated by activation of the IGF-IR.[23] Blocking IGF-IR-mediated signaling by truncated IGF-IR in HT-29 cells inhibits anchorage-independent growth, apoptosis, IGF signaling through Akt-1, and in vivo tumor growth in nude mice.[24]

We have shown that overexpression of IGFBP-3 by stable transfection of IGFBP-3 inhibits Caco-2 cell growth,[25] whereas inhibition of IGFBP-3 expression stimulates growth of these cells.[26] IGFBP-3 enhances p53-dependent apoptosis and differentiation in colonic epithelial cells.[27] In addition to *in vitro* studies, studies with animal models have shown that the number of aberrant crypt foci induced by azoxymethane was significantly lower in IGFBP-3 transgenic mice compared to wild type controls.[28] Furthermore, tumors caused by inoculation of CT26 cells were significantly smaller in BALB/c mice that received IGFBP-3 than in controls. Overexpression of IGFBP-4 also leads to decreased Caco-2 cell growth and increased expression of sucrase-isomaltase, a marker for enterocyte differentiation.[16] These findings are consistent with the hypothesis that IGFBPs inhibit cancer cell growth by binding to endogenously produced IGF-II, thereby preventing IGF-II from interacting with IGF-IR to stimulate cellular proliferation by an autocrine mechanism.

n–3 Polyunsaturated fatty acids (PUFAs)

Available data show that n–3 PUFAs act at different stages of cancer development and through a number of mechanisms such as the inhibition of arachidonic acid-derived prostaglandin production, and Ras and protein kinase C. Consequently, n–3 PUFAs limit tumor cell proliferation, increase apoptosis, support differentiation and possibly limit angiogenesis.[29] We examined the effect of n–3 PUFAs on the proliferation of Caco-2 cells.[30] Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) inhibited cell proliferation compared to linoleic acid (LA). EPA or DHA decreased secretion of both mature 7,500 M_r and higher M_r forms of pro IGF-II compared to LA. Caco-2 cells produced IGFBP-2, IGFBP-4, and IGFBP-6. EPA and DHA increased IGFBP-6 secretion and the steady state levels of IGFBP-6 mRNA compared to LA. Exogenously added IGFBP-6 inhibited Caco-2 cell proliferation. We proposed that low IGF-II/IGFBP-6 ratios may have resulted in less free IGF-II and, as a result, slower proliferation of Caco-2 cells treated with EPA or DHA.

All-trans retinoic acid (tRA)

Retinoids induce growth inhibition and apoptosis in a wide variety of tumor cells.[31] We have shown that treatment of Caco-2 cells with *t*RA inhibited cell growth concomitant with stimulation of IGFBP-6 secretion.[32] 1 μmol/L *t*RA caused a decrease of 48 ± 6% and 70 ± 13% in the concentrations of IGFBP-2 and IGFBP-4, respectively, whereas the concentration of IGFBP-6 increased by 698 ± 20%. *t*RA decreased mRNA levels of IGFBP-2 and IGFBP-4 by 20 ± 3% and 50 ± 8%, respectively, whereas IGFBP-6 mRNA increased by 660 ± 20%. *t*RA did not alter levels of IGF-II mRNA or peptide. Caco-2 cell clones overexpressing IGFBP-6 grew more slowly than vector controls[33] and clones with decreased production of IGFBP-6 grew faster.[32] These results show that the anti-proliferative effect of *t*RA in Caco-2 cells may be due, at least in part, to increased IGFBP-6 expression.

Low-calcemic Vitamin D analogues

Epidemiological and experimental evidence has accumulated indicating that vitamin D and calcium may act as protective agents against colon cancer.[34] In addition to its well defined role in calcium homeostasis, the physiologically active form of vitamin D, 1α,25-dihydroxyvitamin D_3 [1α,25-$(OH)_2D_3$] is also recognized as a potent inhibitor of cancer cells.[35] Since the clinical utilization of 1α,25-$(OH)_2D_3$ in the treatment of cancer is limited by its tendency to cause hypercalcemia,[36] we compared the ability of synthetic analogues of 1α,25-$(OH)_2D_3$, EB1089 and CB1093, to inhibit proliferation of HT-29 cells.[19] 1α,25-$(OH)_2D_3$, EB1089 and CB1093 inhibited cell proliferation, but EB1089 and CB1093 were relatively more potent and had higher efficacies than the native vitamin. Both 10 nM EB1089 and CB1093 markedly inhibited secretion of both mature and higher M_r forms of IGF-II. HT-29 cells secreted IGFBP-4, IGFBP-2, and IGFBP-6. The level of IGFBP-2 was decreased by EB 1089 and CB1093 compared to controls. IGFBP-6 was increased approximately two-fold by EB1089 and CB1093, and exogenously added IGFBP-6 inhibited HT-29 cell proliferation. These results suggest that inhibition of HT-29 cell proliferation by EB1089 and CB1093 may be at least partly attributed to a decreased secretion of IGF-II. The increase in IGFBP-6 concentration coupled with its high affinity for IGF-II may also contribute to decreased cellular proliferation by an indirect mechanism involving sequestration of endogenously produced IGF-II.

Conjugated linoleic acid (CLA)

CLA is comprised of a complex mixture of positional and stereo-isomers of octadecadienoate and has chemoprotective properties in a variety of experimental cancer models.[37] We have demonstrated that a mixture of CLA isomers decreases colon cancer incidence in rats treated with

1,2-dimethylhydrazine.[38] In addition, the two main CLA isomers, *cis*-9,*trans*-11 (*c*9*t*11) and *trans*-10,*cis*-12 (*t*10*c*12), inhibit metastasis of colon cancer cells.[39] Our in vitro studies revealed that CLA decreased growth of HT-29 and Caco-2 cells. In addition, CLA inhibited DNA synthesis and induced apoptosis in HT-29 cells.[40] CLA decreased protein levels of both mature and pro IGF-II and IGF-II transcripts.[41] While exogenous IGF-I and IGF-II produced an increase in cell number, neither IGF-I nor IGF-II counteracted the inhibition of growth due to CLA. CLA decreased IGF-IR transcript and protein levels in a dose-dependent manner. CLA inhibited IGF-I-induced phosphorylation of IGF-IR and IRS-1, recruitment of the p85 regulatory subunit of PI3K to IGF-IR, IGF-IR-associated PI3K activity, and phosphorylated Akt and ERK-1/2 levels. The inhibition of cell proliferation and induction of apoptosis by CLA in HT-29 cells may be mediated in part by its ability to decrease IGF-II synthesis and to down-regulate IGF-IR signalling and the PI3K/Akt and ERK-1/2 pathways.

We compared the individual potencies of *c*9*t*11 and *t*10*c*12 CLA and assessed whether decreased colon cancer cell growth is related to changes in secretion of IGF-II and/or IGFBPs.[42,43] *t*10*c*12 dose-dependently decreased viable cell number, induced apoptosis and decreased DNA synthesis, whereas *c*9*t*11 had no effect. *t*10*c*12 decreased GF-II secretion, whereas c9t11 had no effect. *t*10*c*12 slightly decreased IGFBP-2 production but *c*9*t*11 had no effect. Exogenous IGF-II reversed *t*10*c*12-induced growth inhibition and apoptosis. These results indicate that CLA-inhibited colon cancer cell growth is caused by *t*10*c*12 CLA and may be mediated by decreased IGF-II secretion in colon cancer cells.

CONCLUSIONS

The IGF system has emerged as a potential molecular target for cancer therapy and/or prevention. In recent years, it has been identified as a critical mediator of colon cancer cell growth. As diet and lifestyle modification provide a mean of reducing the risk of developing colon cancer, dietary components which can inhibit IGF-II production and/or IGF-IR signaling hold promise as adjuvant therapies for patients with colon cancer.

The search for individual components in the diet that convey protection and influence the IGF system continues and there is growing interest in other plant-based compounds, so-called phytochemicals, although our understanding of their effects is quite limited at present. Ellagic acid, a natural, dietary phenolic antioxidant induced down-regulation of IGF-II, increased $p21^{waf1/Cip1}$, mediated a cumulative effect on G1/S transition phase and caused apoptotic cell death in SW 480.[17] Future studies are needed to study the mechanisms by which various phytochemicals exhibit chemopreventive effects through regulation of the IGF system.

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AUTHOR DISCLOSURES

Jung Han Yoon Park, no conflicts of interest.

REFERENCES

1. Hassan AB, Macaulay VM. The insulin-like growth factor system as a therapeutic target in colorectal cancer. Ann Oncol. 2002;13:349-56.
2. Singh P, Rubin N. Insulin-like growth factors and binding proteins in colon cancer. Gastroenterology. 1993;105:1218-37.
3. Kaaks R. Nutrition, insulin, IGF-1 metabolism and cancer risk: a summary of epidemiological evidence. Novartis Foundation symposium. 2004;262:247-60; discussion 260-8.
4. Jones JI, Clemmons DR. Insulin-like growth factors and their binding proteins: biological actions. Endocrine Rev. 1995;16:3-34.
5. Dupont J, LeRoith D. Insulin and insulin-like growth factor I receptors: similarities and differences in signal transduction. Horm Res. 2001;55 Suppl 2:22-6.
6. Hwa V, Oh Y, Rosenfeld, RG. The insulin-like growth factor-binding protein (IGFBP) superfamily. Endocr Rev. 1999; 20:761-87.
7. Martin JL. IGFBPs down under. Trends Endocrinol Metab. 2001;12:142-4.
8. Ma J, Pollak MN, Giovannucci, E, Chan, JM, Tao, Y, Hennekens, CH, Stampfer, MJ. Prospective study of colorectal cancer risk in men and plasma levels of insulin-like growth factor (IGF)-I and IGF-binding protein-3. J Natl Cancer Inst. 1999;91:620-5.
9. Palmqvist R, Hallmans G, Rinaldi S, Biessy C, Stenling R, Riboli E, Kaaks R. Plasma insulin-like growth factor 1, insulin-like growth factor binding protein 3, and risk of colorectal cancer: a prospective study in northern Sweden. Gut. 2002;50:642-6.
10. DeLellis K, Rinaldi S, Kaaks RJ, Kolonel LN, Henderson, B, Le Marchand, L. Dietary and lifestyle correlates of plasma insulin-like growth factor-I (IGF-I) and IGF binding protein-3 (IGFBP-3): the Multiethnic Cohort. Cancer Epidemiol Biomarkers Prev. 2004;13:1444-51.
11. Zhang L, Zhou W, Velculescu VE, Kern SE, Hruban RH, Hamilton SR, Vogelstein B, Kinzler KW. Gene expression profiles in normal and cancer cells. Science. 1997;276:1268-72.
12. Winkler R, Delacroix L, Bensbaho K, Lambert S, Collette J, Hodzic D. IGF-II in primary human colorectal tumors: peptide level, activated promoters, parental imprinting and gene rearrangement. Horm Metab Res. 1999;31:148-54.
13. Miraki-Moud F, Jenkins PJ, Fairclough PD, Jordan S, Bustin SA, Jones AM, Lowe DG, Monson JP, Grossman AB, Besser GM, Camacho-Hubner C. Increased levels of insulin-like growth factor binding protein-2 in sera and tumours from patients with colonic neoplasia with and without acromegaly. Clin Endocrinol (Oxf). 2001;54:499-508.
14. Wu Y, Yakar S, Zhao L, Hennighausen L, LeRoith D. Circulating insulin-like growth factor-I levels regulate colon cancer growth and metastasis. Cancer Res. 2002;62:1030-5.
15. Zhang Y, Wick DA, Seetharam B, Dahms NM. Expression of IGF-II and IGF binding proteins in differentiating human intestinal Caco-2 cells. Am J Physiol. 1995;269 (Endocrinol Metabol 32):E804-13.
16. Park JH, Corkins MR, Vanderhoof JA, Caruso NM, Hrbek MJ, Schaffer BS, Slentz DH, McCusker RH, MacDonald RG. Expression of insulin-like growth factor-II and insulin-like growth factor binding proteins during Caco-2 cell proliferation and differentiation. J Cell Physiol. 1996;166:396-406.

17. Narayanan BA, Re GG. IGF-II down regulation associated cell cycle arrest in colon cancer cells exposed to phenolic antioxidant ellagic acid. Anticancer Res. 2001;21:359-64.
18. Guo YS, Jin GF, Townsend CM, Zhang T, Sheng HM, Beauchamp RD, Thompson JC. Insulin-like growth factor-II expression in carcinoma in colon cell lines: implications for autocrine actions. J Am Coll Surg. 1995;181:145-54.
19. Oh YS, Kim EJ, Schaffer BS, Kang YH, Binderup L, MacDonald RG, Park JH. Synthetic low-calcaemic vitamin D(3) analogues inhibit secretion of insulin-like growth factor II and stimulate production of insulin-like growth factor-binding protein-6 in conjunction with growth suppression of HT-29 colon cancer cells. Mol Cell Endocrinol. 2001;183: 141-9.
20. Zarrilli RZ, Romano M, Pignata S, Casola S, Bruni CB, Acquaviva AM. Constitutive insulin-like growth factor-II expression interferes with the enterocyte-like differentiation of Caco-2 cells. J Biol Chem. 1996;271:8108-14.
21. Remacle-Bonnet M, Garrouste F, el Atique F, Marvaldi J, Pommier G. Cell polarity of the insulin-like growth factor system in human intestinal epithelial cells. Unique apical sorting of insulin-like growth factor binding protein-4 in differentiated human colon cancer cells. J Clin Invest. 1995;96: 192-200.
22. Lamonerie T, Lavialle C, Haddada H, Brison O. IGF-2 autocrine stimulation in tumorigenic clones of a human colon- carcinoma cell line. Int J Cancer. 1995;61:587-92.
23. Di Popolo A, Memoli A, Apicella A, Tuccillo C, di Palma A, Ricchi P, Acquaviva AM, Zarrilli R. IGF-II/IGF-I receptor pathway up-regulates COX-2 mRNA expression and PGE2 synthesis in Caco-2 human colon carcinoma cells. Oncogene. 2000;19:5517-24.
24. Adachi Y, Lee CT, Coffee K, Yamagata N, Ohm JE, Park KH, Dikov MM, Nadaf SR, Arteaga CL, Carbone DP. Effects of genetic blockade of the insulin-like growth factor receptor in human colon cancer cell lines. Gastroenterology. 2002;123:1191-204.
25. MacDonald RG, Schaffer BS, Kang IJ, Hong SM, Kim EJ, Park JH. Growth inhibition and differentiation of the human colon carcinoma cell line, Caco-2, by constitutive expression of insulin-like growth factor binding protein-3. J Gastroenterol Hepatol. 1999;14:72-8.
26. Park JHY. Growth stimuation and inhibition of differentiation of the human colon carcinoma cell line Caco-2 with an anti-sense insulin-like growth factor binding protein-3 construct. J Biochem Mol Biol. 1999;32:266-72.
27. Williams AC, Collard TJ, Perks CM, Newcomb P, Moorghen M, Holly JM, Paraskeva C. Increased p53-dependent apoptosis by the insulin-like growth factor binding protein IGFBP-3 in human colonic adenoma-derived cells. Cancer Res. 2000;60:22-7.
28. Kirman I, Poltoratskaia N, Sylla P, Whelan RL. Insulin-like growth factor-binding protein 3 inhibits growth of experimental colocarcinoma. Surgery. 2004;136:205-9.
29. Roynette CE, Calder PC, Dupertuis YM, Pichard C. n–3 polyunsaturated fatty acids and colon cancer prevention. Clin Nutr. 2004;23:139-51.
30. Kim EJ, Kim WY, Kang YH, Ha YL, Bach L, Park JHY. inhibition of Caco-2 cell proliferation by (n–3) fatty acids: possible mediation by increased secretion of insulin-like growth factor binding protein-6. Nutr Res. 2000;20:1409-21.
31. Hong WK, Itri LM. Retinoids and human cancer. in *The Retinoids* (eds. Sporn MB, Roberts AB & Goodman, D.S.) 597-630 (Raven Press, New York, 1994).
32. Kim EJ, Schaffer BS, Kang Y-H, Macdonald RG, Park JHY. Decreased production of insulin-like growth factor binding protein (IGFBP)-6 by transfection of colon cancer cells with an antisense IGFBP-6 cDNAconstruct leads to stimulation of cell proliferation. J Gastroenterol Hepatol. 2002;17:563-70.
33. Kim EJ, Kang YH, Schaffer BS, Bach LA, MacDonald RG, Park JH. Inhibition of Caco-2 cell proliferation by all-trans retinoic acid: role of insulin-like growth factor binding protein-6. J Cell Physiol. 2002;190:92-100.
34. Bostick RM, Potter JD, Sellers TA, McKenzie DR, Kushi LH, Folsom AR. Relation of calcium, vitamin D, and daily food intake to incidence of colon cancer among older women. Am J Epidemiol. 1993;137:1302-17.
35. Bikle DD. Clinical counterpoint: vitamin D: new actions, new analogs, new therapeutic potential. Endocrine Rev. 1992;13:765-84.
36. Feldman D, Zhao X-Y, Krishnan AV. Editorial/mini-review: vitamin D and prostate cancer. Endocrinology. 2000;141:5-9.
37. Belury MA. Inhibition of carcinogenesis by conjugated linoleic acid: potential mechanisms of action. J Nutr. 2002; 132:2995-8.
38. Park HS, Ryu JH, Ha YL, Park JH. Dietary conjugated linoleic acid (CLA) induces apoptosis of colonic mucosa in 1,2-dimethylhydrazine-treated rats: a possible mechanism of the anticarcinogenic effect by CLA. Br J Nutr. 2001;86:549-55.
39. Soel SM, Choi OS, Bang MH, Yoon Park JH, Kim WK. Influence of conjugated linoleic acid isomers on the metastasis of colon cancer cells in vitro and in vivo. J Nutr Biochem. 2007;18:650-7.
40. Cho HJ, Kim WK, Kim EJ, Jung KC, Park S, Lee HS, Tyner AL, Park JH. Conjugated linoleic acid inhibits cell proliferation and ErbB3 signaling in HT-29 human colon cell line. Am J Physiol Gastrointest Liver Physiol. 2003;284:G996-1005.
41. Kim EJ, Kang IJ, Cho HJ, Kim WK, Ha YL, Park JH. Conjugated linoleic acid downregulates insulin-like growth factor-I receptor levels in HT-29 human colon cancer cells. J Nutr. 2003;133:2675-81.
42. Kim EJ, Holthuizen PE, Park HS, Ha YL, Jung KC, Park JH. Trans-10,cis-12-conjugated linoleic acid inhibits Caco-2 colon cancer cell growth. Am J Physiol Gastrointest Liver Physiol. 2002;283:G357-67.
43. Cho HJ, Lee HS, Chung CK, Kang YH, Ha YL, Park HS, Park JH. trans-10, cis-12 conjugated linoleic acid reduces insulin-like growth factor-II secretion in HT-29 human colon cancer cells. J Med Food. 2003;6:193-9.