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Anti-Aging Therapy with Human Growth Hormone Associated with Metastatic Colon Cancer in a Patient with Crohn's Colitis

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Abstract

Background—The non-approved use of human growth hormone (HGH) for ‘anti-aging’ has been increasing. Theoretical concerns for neoplastic potentiation by HGH have been raised, but not clinically proven.

Methods—We report the case of a 68-year-old man with colonic Crohn's disease who was found to have aggressive, metastatic colon cancer. The patient had been receiving HGH therapy for anti-aging purposes for 7 years prior to presentation. Posthumously, normal and malignant colonic tissue was examined for qualitative and quantitative molecular profiles of growth hormone (GH) and its signaling molecules, using immunohistochemistry and RNA extraction with PCR amplification.

Results—Immunoreactivity was more robust in tumor tissue than in normal colon for insulin-like growth factor-1 receptor (IGF1R) but not for IGF, GH, or GH-receptor (GHR). RNA extraction with quantitative PCR showed that IGF-1R and vascular endothelial growth factor (VEGF) expression, but not IGF-1, GHR or suppressor of cytokine signaling-2 (SOCS2), were higher in tumor than in normal colonic tissue.

Conclusions—To our knowledge, colorectal cancer development concurrent with administration of HGH for anti-aging purposes has not previously been reported. That it occurred in an individual already at increased risk for colon cancer underscores the need for further investigation of the pro-neoplastic potential of growth hormone supplementation for anti-aging.

Introduction

The use of GH in adults is approved for patients with demonstrated pituitary deficiency, or those with AIDS-associated wasting.¹ Approximately 100,000 individuals obtain GH for anti-aging purposes annually in the United States without a prescription, and about 30% of GH prescriptions in the United States are for off-label use such as anti-aging.^{2, 3} Due to the potential benefits of GH administration on bone density, muscle mass, and cardiovascular health in GH deficient individuals, the practice of administering GH to healthy adults has

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No Conflicts of Interest Exist

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become increasingly common owing to claims of ‘anti-aging’ properties. However, a recent metaanalysis of GH administration in healthy adults showed no important benefit to body composition and that side effects were common.⁴ Furthermore, concerns have been raised regarding the carcinogenic potential of HGH.⁵ Several epidemiologic studies have reported an association between increased IGF1 serum levels and the risk of colorectal cancer.^{6–8} Acromegaly, a condition of endogenous GH excess has been reported to be associated with increased risk of neoplasia including colorectal adenomas and carcinoma,^{9, 10} and guidelines for colorectal cancer screening in these individuals have been published.¹¹

Despite the growing off-label use of HGH for ‘anti-aging’ and its potential carcinogenic risk, there is a paucity of published reports attributing its use to the occurrence of cancer in this setting. We describe a case of metastatic colon cancer in a patient with longstanding Crohn’s disease who was receiving daily anti-aging treatment with HGH for years prior to the diagnosis of cancer. We performed molecular assessment for the presence of IGF-1, IGF-1R, GH, GHR in malignant and non-malignant colonic tissue. We also performed molecular analysis for suppressor of cytokine signaling (SOCS)-2, a GH-dependent negative signaling molecule implicated in the regulation of intestinal epithelial cell growth¹² and vascular endothelial growth factor (VEGF), an IGF1-dependent angiogenic gene that promotes colon cancer growth.^{13, 14}

Case Report

A 68-year-old man with longstanding colonic Crohn’s disease (CD) was admitted with hematochezia and abdominal pain. He was receiving prednisone 6 mg daily and 5-aminosalicylates, and had never taken immunomodulator or biologic therapy. For 7 years prior to admission, the patient sought therapy at anti-aging clinics, and was maintained with testosterone 200 mg weekly injection, DHEA 100 mg daily, and recombinant human growth hormone (HGH) (0.6 mg subcutaneously twice daily).

Colonoscopy identified a 7-cm cecal mass, and laparotomy revealed widely metastatic colon cancer. The patient had undergone 2 colonoscopies within 2 years of presentation, both of which showed changes consistent with chronic, active colitis in the right colon, but no definite dysplasia or malignancy; it is not known whether the patient had ever had a colonoscopy for dysplasia screening. At the time of admission, carcinoembryonic antigen level was 38 ng/mL (normal <3 ng/mL) and insulin-like growth-factor-1 (IGF1) was 245 ng/mL (normal 36–237 ng/mL). The patient succumbed to his illness within 5 months of diagnosis. Posthumously, normal and malignant colonic tissue was examined for qualitative and quantitative molecular profiles of growth hormone (GH), GH receptor, IGF1, IGF1-receptor (IGF1R), suppressor of cytokine signaling (SOCS)2 and vascular endothelial growth factor (VEGF)-A using immunohistochemistry and RNA extraction with PCR amplification.

Methods

Human Subjects Approval

Permission to review medical records and to obtain normal and malignant colonic tissue from archived formalin-fixed, paraffin-embedded sections from laparotomy for molecular investigation was obtained from the institutional review board at Cedars-Sinai Medical Center, Los Angeles, CA after the patient’s demise.

Immunohistochemistry

Paraffin slices (5 microns) of resected malignant and normal tissue were deparaffinated and rehydrated, and antigens retrieved by microwave heating. Processed slices were blocked with

5% fetal bovine serum and 0.6% Tween 20 in buffered saline, and incubated with primary antibody at 1:100 for 2 hours followed by rhodamine-labeled goat anti-mouse IgG or donkey anti-rabbit IgG at 1:200 for 1 hour. Slices were counterstained with Hoechst 33342 at 1:5000. Stained slices were preserved in Prolong Gold anti-fade reagent (Invitrogen, Carlsbad, CA). Antibodies used included rabbit polyclonal anti-GH (Dako, Carpinteria, CA), mouse monoclonal anti-GHR (MAB263, Abcam, Cambridge, MA), rabbit polyclonal anti-IGF-1 (H-70, Santa Cruz Biotechnology, Santa Cruz, CA), and rabbit polyclonal anti-IGF-1R (C-20, Santa Cruz). Sections were observed with a Nikon Eclipse TE200 fluorescence microscope, and pictures acquired with a SPOT digital camera (Diagnostic Instruments, Inc. Sterling Heights, MI).

RNA extraction and Q-PCR

RNA extraction was performed from 10µm thick sections of freshly cut normal and tumor formalin-fixed, paraffin-embedded (FFPE) tissue, respectively, using RNeasy® FFPE Kit (Qiagen). Reverse transcription of 1.0 µg total RNA was performed with M-MLV-Reverse Transcriptase (Invitrogen). Q-PCR was performed with SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA) in a 20 µl reaction system, containing 10 µl Master Mix, 500 nM primer (each) and appropriate DNA aliquots and analyzed with Bio-Rad iQ5 Optical System Software. To allow the use of fragmented RNA, the following primers were specifically designed to amplify small amplicons: GH (Genebank Accession NM_000515), sense 5'-CAGGAGTGTCTTCGCCAACA-3', anti-sense 5'-TCCCCATCAGCGTTTGGAT-3' (amplicon 101 nt); GHR (Genebank Accession NM_000163), sense 5'-CAACCAGATCCACCCATTGC-3', anti-sense 5'-GAATCCCAGTTAAACTGACGTTTCAG-3' (amplicon 61 nt); IGF-I (Genebank Accession NM_000618), sense 5'-GCTGGTGGATGCTCTTCAGTT-3', anti-sense 5'-CCCTGTGGGCTTGGTGAAT-3' (amplicon 64 nt); IGF-IR (Genebank Accession NM_000875), sense 5'-ACGTGAAGATCCGCCATTCT-3', anti-sense 5'-CCTAGGATGAGGCGAAGGTTT-3' (amplicon 70 nt); SOCS2 (Genebank Accession NM_003877), sense 5'-GCAAGGATAAGCGGACAGGTC-3', anti-sense 5'-AGAGCGGTTTGGTCAGATAAAGG-3' (amplicon 72 nt); VEGF-A (Genebank Accession NM_001025366), sense 5'-GCTTGCCATTCCCCACTTG-3', anti-sense 5'-TTTCTCTTTCTGCTGGTTTCC-3' (amplicon 97 nt) and β-actin (Genebank Accession BC002409), sense 5'-TTGAATGATGAGCCTTCGTG-3', anti-sense 5'-GCCTTCATACATCTCAAGTTGG-3' (amplicon 67 nt).

Statistical analysis

Results are expressed as mean ± SD and differences were evaluated using Student's t test (statistical significance was set at p<0.05).

Results

Light histology showed distinct areas of normal colon and poorly differentiated adenocarcinoma (not shown). Immunoreactivity was more robust in tumor tissue than in normal colon for IGF1R, but not for IGF, GH, or GHR (Figure 1). When RNA was subjected to Q-PCR, IGF-1R and VEGF expression were shown to be higher in the tumor than in normal tissue. However, IGF1, GHR, and SOCS2 expression were not elevated (Figure 2).

Discussion

The attributable risk of cancer in patients receiving GH therapy has not been definitively established. In a metanalysis evaluating the reported incidence of neoplasia in individuals with GH deficiency treated with exogenous GH, there was a modest but significant increased

incidence of overall cancer mortality, colorectal cancer, and Hodgkin's disease.¹⁵ However, surveillance studies of GH replacement for GH-deficient adults have not shown enhanced cancer incidence or mortality. The potential risk of adenomatous polyps and cancer death in the setting of excess GH may be due to increased serum insulin like growth factor-I (IGF-I) which can stimulate cell growth and inhibit apoptosis.¹⁶ IGF-I receptors are expressed on human colorectal cancer cells and IGF-I is known to be a stimulator of colorectal cancer cell growth *in vitro*.¹⁷ IGF1R has also been shown to play an important role in angiogenesis and growth of human colon cancer cells.¹⁸ Our findings of increased IGF1R and VEGF expression supports the notion that VEGF acts to promote tumor angiogenesis *via* IGF1 signalling.¹³ Despite our inability to detect SOCS2 in either normal or malignant tissue, SOCS2 may yet be implicated in this patient's cancer, as haplotype insufficiency for SOCS2 promotes colonic polyposis in the setting of excess GH in mice.¹⁹

In this case report, the patient had longstanding Crohn's colitis, a known risk factor for colorectal cancer. The degree to which exogenous HGH anti-aging treatment may have contributed to malignant transformation of colonic epithelium or accelerated growth of a pre-existing neoplasm cannot be determined on the basis of our findings. However, 2 colonoscopic evaluations within 2 years of presentation did not detect malignancy or dysplasia, suggestive of an accelerated process of malignant transformation and/or growth. IGF1, which was elevated in this patient's serum at the time of admission, has been shown to both inhibit apoptosis and allow progression through the cell cycle, which could potentiate both malignant transformation as well as accelerated growth of an existing neoplasm.²⁰ Conversely, inflammatory bowel disease has been characterized by GH resistance and relative IGF-1 deficiency,²¹ a finding which has prompted clinical investigations for the use of hGH and other growth factors for the treatment of both CD and ulcerative colitis. A randomized, placebo-controlled study showed significant short-term improvement in adults with CD treated with hGH.²² However, concerns about malignant transformation of chronically inflamed, rapidly dividing colonic epithelial tissue in response to growth factors have been raised.²³

Our findings of increased tumor tissue IGF1R expression as compared with normal colon lends support to an etiologic role for the GH/IGF1 axis in the development and progression of colon cancer, as has been previously described.^{24, 25} However, our findings do not discriminate between accelerated growth influenced by increased local GH signaling or increased circulating IGF-1. Furthermore, the molecular basis of colitis-related colorectal cancer is thought to involve multiple genetic alterations, including mutations in p53, APC, and *k-ras*.²⁶ The assessment of these markers and description of the complex interplay between these molecular alterations and endocrine and paracrine signals is important to understanding the molecular pathogenesis of cancer in this patient, but beyond the scope of this report.

To our knowledge, colorectal cancer development concurrent with administration of HGH for anti-aging purposes has not previously been reported. That it occurred in an individual already at increased risk for colon cancer with inflammatory bowel disease underscores the need for further investigation of the pro-neoplastic potential of growth hormone supplementation in individuals for 'anti-aging', as its use for this non-approved indication becomes more widespread.

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Figure 1.

Immunofluorescent staining of growth hormone (GH), growth hormone receptor (GHR), insulin-like growth factor-1 (IGF-1), and insulin-like growth factor-1 receptor (IGF-1R) in normal colon mucosa and colon carcinoma. Paraffin sections of normal colon mucosa and colon carcinoma from the patient were stained for GH, GHR, IGF-1, or IGF-1R (Red) and counter-stained with Hoechst 33342 for nuclei (Blue). Figure 1A shows increased cytoplasmic signal for IGF-1R, but not for GH, GHR, or IGF-1, in tumor relative to normal colon tissue. Figures 1B and 1C show merged images for IGF1R staining in normal and malignant colonic tissue, respectively.

Figure 2.

Increased expression of IGF-1R and VEGF, but not GHR or IGF-1, in tumor relative to normal colon tissue as determined by quantitative RT-PCR; **, $p < 0.01$.