Short Communication

Insulin-Like Growth Factor Axis and Oncogenic Human Papillomavirus Natural History

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Abstract

High serum levels of insulin-like growth factor-I (IGF-I) are reported to be a risk factor for several common cancers, and recent cross-sectional data suggest a possible additional association of IGF-I with cervical neoplasia. To prospectively assess whether circulating IGF-I levels influence the natural history of oncogenic human papillomavirus (HPV), the viral cause of cervical cancer, we conducted a pilot investigation of 137 women who underwent semiannual type-specific HPV DNA PCR testing and cervical cytology. Total IGF-I and IGF binding protein-3 (IGFBP-3), the most abundant IGFBP in circulation, were measured using baseline serum specimens. Having a high IGF-I/IGFBP-3 ratio was associated with increased persistence of oncogenic HPV infection [that is, a lower rate of clearance; adjusted hazard ratio (AHR), 0.14; 95% confidence interval (95% CI), 0.04-0.57], whereas IGFBP-3 was inversely associated with both the incident detection of oncogenic HPV (AHR, 0.35; 95% CI, 0.13-0.93) and the incidence of oncogenic HPV-positive cervical neoplasia (that is, squamous intraepithelial lesions at risk of progression; AHR, 0.07; 95% CI, 0.01-0.66). These prospective data provide initial evidence that the IGF axis may influence the natural history of oncogenic HPV. (Cancer Epidemiol Biomarkers Prev 2008;17(1):245–8)

Introduction

Insulin-like growth factor-I (IGF-I), a peptide hormone, has mitogenic and antiapoptotic activities. IGF-I mediates many of the effects of growth hormone, and most cells throughout the body express the IGF-I receptor. High levels of IGF-I in circulation are reported to be positively associated with risk of several common tumors, including colorectal and breast cancers (1, 2), and recent data suggest a possible additional role of IGF-I in cervical tumorigenesis. *In vitro* studies, for example, found that (*a*) IGF-I is a potent stimulator of cervical cancer cell invasiveness (3), (*b*) antibody to the IGF-I receptor is an inhibitor of cervical cancer cell proliferation (4), (*c*) down-regulation of the IGF-I receptor can reverse the transformed phenotype of cervical cancer cell lines (5), and (*d*) the E7 oncoprotein of human papillomavirus (HPV) 16, the HPV type associated with half of all cervical cancers, binds and inactivates IGF binding protein (IGFBP)-3, the most abundant IGFBP in circulation (evidence that certain HPV may target the IGF axis; ref. 6).

Two recent cross-sectional epidemiologic studies reported additional evidence of an association between the IGF axis and cervical neoplasia. The first study reported a significant association of cervical neoplasia with high serum IGF-I levels and with a high IGF-I/IGFBP-3 molar ratio (considered an indirect measure of free, presumably bioactive, IGF-I; ref. 7). The second study reported similar positive associations with high levels of IGF-II (a related growth factor) and low levels of

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IGFBP-3 (8). However, two other cross-sectional studies found conflicting results, that is, an inverse association between IGF-I and cervical neoplasia (9, 10).

Prospective studies may be essential to the accurate assessment of the IGF axis and its effects on oncogenic HPV and cervical neoplasia [that is, squamous intraepithelial lesions (SIL)], helping to distinguish these effects from their converse (the effect of HPV and neoplasia on the IGF axis; that is, reverse causality). We, therefore, conducted a pilot investigation to evaluate the associations of serum IGF-I and IGFBP-3 levels with incident detection and persistence of oncogenic HPV as well as the development of SIL. Based on the associations of IGF-I with other epithelial tumors and the proliferative effects of IGF-I on cervical cancer cells *in vitro*, we hypothesized that IGF-I would have a positive association and IGFBP-3 would have a negative association with HPV infection and SIL.

Materials and Methods

Subjects and Specimens. Between October 1994 and November 1995, 568 HIV-seronegative women were enrolled as a comparison group for HIV-seropositive subjects in the Women's Interagency HIV Study through similar clinical and outreach sources in six U.S. cities (11). All subjects provided informed consent and each local institutional review board provided approval for the study. On an ongoing basis, the Women's Interagency HIV Study conducts semiannual study visits during which serum is obtained, and a cervicovaginal lavage specimen is collected for HPV DNA testing followed by a Papanicolaou smear (12-14). All Papanicolaou smears were interpreted centrally using the 1991 Bethesda System criteria (15).

For the current study, 150 HIV-seronegative women were randomly selected for IGF-I and IGFBP-3 testing from the HIV-seronegative cohort; subjects who missed sequential visits during the first 2 years of follow-up were excluded. Of those selected, 146 women had baseline serum specimens available for testing. Nine reported having had a hysterectomy before enrollment. Thus, 137 HIV-seronegative women contributed data to our analysis. HIV-seropositive women were not studied in this initial pilot investigation because of concerns regarding confounding (that is, IGF-I and IGFBP-3 may be associated with both cervical HPV and HIV disease), which would have complicated the interpretation of the data.

Laboratory Methods. HPV DNA testing was conducted using a well-established PCR assay that employed MY09/MY11/HMB01 L1 consensus primers as reported previously (12-14). Amplification of a 268-bp cellular β-globin DNA fragment was included in each assay as an internal control. Following amplification, the presence of HPV DNA sequences was assessed using filters individually hybridized with biotinylated oligonucleotide probes for >35 specific HPV types as well as a general probe mixture able to detect most anogenital HPV DNA types. HPV types 16/18/31/33/35/39/45/51/52/56/58/59/68/73/82 were considered oncogenic (16-18). All other HPV detected were considered nononcogenic.

All serum specimens were stored at -70°C until tested for total IGF-I and IGFBP-3 levels using enzyme linked immunosorbent assays from DSL, in accordance with manufacturer's recommendations. We note that in HIV-seronegative women IGF-I and IGFBP-3 levels are known to be stable for periods measured in years (19), and it is common practice in prospective cancer epidemiology studies to use a single baseline (time-independent) value to assess risk of disease during as much as 5 to 10 or more years of follow-up (1).

Statistical Methods. IGF-I and IGFBP-3 data were initially divided into quartiles, in keeping with what has commonly been done in prior epidemiologic studies of IGF-I and cancer (the serocutoffs are shown in the footnote to Table 1; ref. 1). Because of the small size of this pilot study, however, we dichotomized these categorical data into high (quartiles 2-4) and low (quartile 1) values. To measure the associations of total IGF-I and IGFBP-3 with prevalent detection of oncogenic HPV DNA and SIL, we used multivariate logistic regression models that incorporated generalized estimating equations to adjust the SEs for repeated measures (that is, across multiple visits and multiple HPV types) as described previously (13, 14). IGF-I and IGFBP-3 levels remain stable for periods measured in years (see Laboratory Methods) and were incorporated as time-independent variables in our analyses. To study incident

Table 1. Associations of total IGF-I, IGFBP-3, and IGF-I/IGFBP-3 molar ratio with the prevalence, incident detection, and time to clearance of oncogenic HPV and the prevalence and incidence of SIL in oncogenic HPV-positive women (oncogenic HPV-positive SIL)

	Incident detection of oncogenic HPV*	Clearance of oncogenic HPV*	Prevalence of oncogenic HPV †	Incident detection of oncogenic HPV-positive SIL*	Prevalence of oncogenic HPV-positive SIL †
Total IGF-I	1.08 (0.44-2.69)	0.39 (0.09-1.64)	1.68 (0.75-3.79)	1.22 (0.11-15.72)	2.37 (0.33-17.07)
IGFBP-3	0.35 (0.13-0.93) ‡	0.72 (0.34-1.53)	0.63 (0.24-1.61)	0.07 (0.01-0.66) ‡	0.61 (0.09-4.15)
IGF-I/IGFBP-3§	1.58 (0.65-3.80)	0.14 (0.04-0.57) ‡	1.04 (0.53-2.03)	0.81 (0.09-7.34)	1.93 (0.47-7.98)

NOTE: Number of events: prevalent oncogenic HPV (94), incident oncogenic HPV (40), oncogenic HPV clearance (55 including 18 incident infections and 37 prevalent infections), prevalent oncogenic SIL (18), and incident oncogenic SIL (6). Quartile serocutoffs for IGF-I were 174, 219, and 275 ng/mL. Serocutoffs for IGFBP-3 were 2,497, 3,094, and 3,661 ng/mL.

*HR comparing quartiles 2 to 4 with quartile 1 (95% CI) adjusted for age (≤29, 30-39, and ≥40 y), race (White, Black, and Hispanic or other), and number of male sexual partners within the last 6 months (0, 1 and married, 1 and single, and ≥2).

† Odds ratio comparing quartiles 2 to 4 with quartile 1 (95% CI) adjusted for age (≤29, 30-39, and ≥40 y), race (White, Black, and Hispanic or other), and number of male sexual partners within the last 6 months (0, 1 and married, 1 and single, and ≥2).

‡ $P < 0.05$.

§Ratio of total IGF-I to IGFBP-3.

detection of HPV, we used Cox proportional hazards regression models that adjusted for the multiplicity of HPV types by employing the Wei Lin Weissfeld method as in prior studies (14).

Cox models were also employed to assess the associations of IGF-I and IGFBP-3 with type-specific HPV persistence (that is, time to clearance, with clearance defined as the first negative type-specific PCR result following initial detection of a given HPV type). We note that in prior Women's Interagency HIV Study analyses the results for HPV persistence were unaltered by defining clearance either as the first negative result or as two negative results at sequential visits (14). In any event, this pilot investigation lacks sufficient data to assess the latter, more stringent, definition. The persistence model was stratified by whether the infection was prevalent at baseline or first detected during follow-up (that is, incident detection) as described previously (20). Similar logistic and Cox models were also used to study the associations of IGF-I and IGFBP-3 with SIL. All models were adjusted for age, race/ethnicity, and the number of male sexual partners within the last 6 months (see the footnote to Table 1 for details). Women who underwent treatment for cervical neoplasia or had a hysterectomy during follow-up were censored at the visit before the procedure.

Results

The 137 HIV-seronegative women included in this pilot study had a baseline mean age of 34 years, were predominantly Black (61%) or Hispanic (23%), and had a mean and median number of sexual partners in the last 6 months of 1.4 and 1.0, respectively. The median period of follow-up for HPV DNA was 5.5 years. Each woman contributed multiple observations because of repeated HPV and cytologic testing over time and the existence of multiple different HPV (the number of incident, prevalent, and clearance events are shown in the footnote to Table 1). Statistical methods appropriate for such data were employed.

Table 1 shows the associations of IGF-I and IGFBP-3 with the natural history of oncogenic HPV and the development of SIL. In multivariate Wei Lin Weissfeld Cox analysis, IGFBP-3 had a statistically significant inverse association with risk of incident detection of oncogenic HPV [adjusted hazard ratio (AHR), 0.35; 95% confidence interval (95% CI), 0.13-0.93] and with the incidence (AHR, 0.07; 95% CI, 0.01-0.66) of oncogenic HPV-positive SIL (SIL at risk of progression) in models that adjusted for age, race, and the number of recent sex partners. A high IGF-I/IGFBP-3 molar ratio was associated with reduced clearance (that is, greater persistence) of oncogenic HPV (AHR, 0.14; 95% CI, 0.04-0.57) in similar models. No other associations with oncogenic HPV detection were statistically significant, although the findings showed a pattern fairly consistent with those above.

For example, high total IGF-I was nonsignificantly associated with greater prevalence of oncogenic HPV and with oncogenic HPV-positive SIL, whereas high IGFBP-3 had a nonsignificant inverse association with these same endpoints. Inclusion of IGF-I and IGFBP-3 in the same models had no affect on the above results. For nononcogenic HPV, we observed only one significant finding, an unexpected inverse association of IGF-I (odds ratio, 0.50; 95% CI, 0.28-0.90) with prevalent detection of a nononcogenic HPV type. No other associations of IGF-I and IGFBP-3 with nononcogenic HPV approximated statistical significance, although the number of events was similar to the number observed with oncogenic HPV (data not shown).

Discussion

The findings of this small pilot study are consistent with our hypothesis that the IGF axis may influence the natural history of HPV infection and the development of cervical neoplasia. Specifically, women with high serum IGFBP-3 levels had significantly lower rates of incident oncogenic HPV detection, and lower incidence of oncogenic HPV-positive SIL, than women with low serum IGFBP-3 levels. The IGF-I/IGFBP-3 molar ratio, in contrast, was positively associated with persistence of oncogenic HPV infection. We note that these prospective data are in keeping with two prior cross-sectional studies. The first study reported that cervical neoplasia was positively associated with the IGF-I/IGFBP-3 ratio and total IGF-I (7), and the second study reported that cervical neoplasia was positively associated with IGF-II (a related growth factor) but inversely associated with IGFBP-3 (8). Although in the current pilot investigation none of the additional associations of oncogenic HPV with IGF-I or IGFBP-3 were statistically significant, they were fairly similar to the above: total IGF-I had a nonsignificantly positive association with prevalence of oncogenic HPV and oncogenic HPV-positive SIL, whereas high IGFBP-3 had a nonsignificant inverse association with these same endpoints.

Laboratory studies have also reported data consistent with a positive effect of IGF-I and an inhibitory effect of IGFBP-3 on cervical tumorigenesis. For example, *in vitro* data indicate that the inhibitory effects of IGFBP-3 are likely due to both its sequestration of IGF-I and IGF-I-independent effects; specifically, IGFBP-3 can bind proteins involved in cell cycle in the nucleus (21) and has antiproliferative (22-24) and direct proapoptotic activities (25-28). Thus, high IGFBP-3 levels could lead to less replication and/or greater loss of oncogenic HPV infected cells (that is, to levels below the threshold of detection). A positive association of IGF-I/IGFBP-3 molar ratio with persistence of oncogenic HPV infection was also expected. This ratio is used by some investigators as an indirect measure of free (presumably bioactive) IGF-I levels; indeed, in a recent study of postmenopausal women, we found a modest correlation of ~20% between the total IGF-I/IGFBP-3 ratio and free IGF-I levels.[10] Therefore, the positive association of total IGF-I/IGFBP-3 ratio with persistent infection by oncogenic HPV could reflect the antiapoptotic effects of free/bioactive IGF-I. Alternatively, an association of IGF-I/IGFBP-3 molar ratio with persistent oncogenic HPV infection could reflect the relative balance of the competing effects of IGF-I (mitogenic/antiapoptotic) and IGFBP-3 (antimitotic/proapoptotic) on infected cells.

[10] H.D. Strickler, personal communication.

Two additional cross-sectional studies found inverse associations of IGF-I with the presence of cervical neoplasia (9, 10), although in their careful discussion Schaffer et al. (the most recent of the two studies) point to the difficulty in explaining such results from a biological perspective (10). There was also one inverse association with IGF-I in our study: high total IGF-I was associated with lower (not higher) prevalence of nononcogenic HPV. However, no other associations of IGF-I or IGFBP-3 with nononcogenic HPV approximated significance nor was the inverse association we observed actually consistent with Schaffer et al. Their study showed inverse associations of IGF-I with two oncogenic (in contrast to nononcogenic) HPV. Therefore, although we cannot exclude the possibility of different associations of the IGF axis with oncogenic and nononcogenic HPV, given the small size of this pilot study, we feel the inverse association of nononcogenic HPV detection with IGF-I was most likely a spurious finding.

Taken as a whole, this small prospective study provides initial evidence that the IGF axis may affect the natural history of oncogenic HPV. A larger, more comprehensive prospective investigation is indicated to better clarify these relationships, including any potential HPV type-specific differences in the effects of IGF-I and IGFBP-3. If specific components of the IGF axis can affect the incidence and persistence of oncogenic HPV, then the IGF axis may be a viable target for the development of novel therapeutic interventions to disrupt this cancer pathway.

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