Signatures of cervicovaginal inflammation in patients over the course of cervical carcinogenesis

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Abstract

Infection with high-risk types of human papilloma virus (HPV) is the primary cause of invasive cervical cancer (ICC). However, the cause of persistent HPV infection and progression to invasive cervical carcinoma (ICC) remains unclear. Mucosal inflammation and a dysbiotic vaginal microbiome (VMB) can cause epithelial barrier breach and increase infection. Additionally, mucosal inflammation and/or a dysbiotic VMB also could promote tumor development and progression to invasive disease. We collected cervicovaginal lavages (CVL) and swabs from premenopausal women with histologically confirmed low (LGD) and high grade cervical dysplasia (HGD), ICC, or healthy controls. Mean age was 38 years with 45% Hispanic. Over 80 CVLs were comprehensively quantified for 34 secreted immune mediators with a variety of immune functions, including proinflammatory, hematopoietic, adaptive immunity, immunoregulation, antimicrobial peptides (AMP), protease inhibitors, iron-limiting proteins and cancer biomarkers using Luminex assays. Patients with ICC, but not LGD or HGD exhibited significantly increased levels of proinflammatory mediators and cancer biomarkers relative to controls. We observed a significantly increased trend across groups for specific targets. Furthermore, AMP levels exhibited a decreased trend as cervical dysplasia progressed to ICC. Ongoing VMB sequencing will allow us to identify microbial drivers and compare those vaginal communities to the level of mucosal inflammation. Our study revealed unique immune signatures in the microenvironment associated with cervical carcinogenesis. This report is an initial step to understand the complex interplay between mucosal inflammation, epithelial barrier function and vaginal microbiome in driving cervical carcinogenesis.

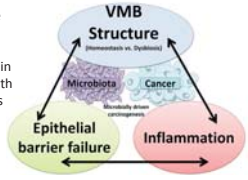

Figure 1. Proposed model.

Patient demographics

Table 1. The statistical significance in patient demographics between groups. The differences in the demographic variables between groups were tested using analysis variance model (ANOVA) for continuous variables and Fisher's exact test for categorical variables.

	n	Control HPV- (n=20)	Control HPV+ (n=31)	LGD (n=12)	HGD (n=27)	ICC (n=10)	*P* value
Age: mean (SD)	100	39.55 (7.35)	37.64 (9.38)	35.08 (7.24)	38.29 (8.46)	38.90 (9.09)	0.68
Ethnicity: n (%)							
Hispanic	47	5 (25.00)	14 (45.16)	7 (58.33)	17 (62.96)	4 (40.00)	
Non-Hispanic	53	15 (75.30)	17 (54.84)	5 (41.67)	10 (37.04)	6 (60.00)	0.11
pH: n (%)							
≤ 4.5	20	9 (45.00)	6 (22.22)	3 (27.27)	2 (7.41)	0 (0.00)	
> 4.5	74	11 (55.00)	21 (77.78)	8 (72.73)	25 (92.59)	9 (100.00)	**0.01**
BMI: mean (SD)	100	32.25 (11.20)	28.53 (7.15)	27.41 (4.56)	30.70 (7.66)	27.10 (6.96)	0.28
BMI: n (%)							
≤ 25	34	7 (35.00)	12 (38.71)	4 33.33)	8 (29.63)	3 (30.00)	
>25	66	13 (65.00)	19 (61.29)	8 (66.67)	19 (70.37)	7 (70.00)	0.96

Vaginal pH and ethnicity

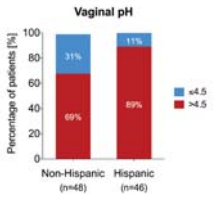

Figure 2. Hispanic women exhibit significantly higher vaginal pH compared to Non-Hispanic Caucasian women. The graph shows percentage of patients with normal pH (≤4.5) indicated in blue and abnormal pH level (>4.5) indicated in red. The differences in the pH between groups were determined using Fisher's exact test (p=0.02).

Vaginal pH during cervical cancer progression

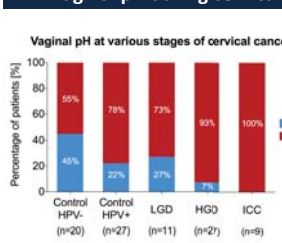

Figure 3. Vaginal pH significantly increases during cervical cancer progression. The graph shows percentage of patients with normal pH (≤4.5) indicated in blue and abnormal pH level (>4.5) indicated in red. The differences in the pH between groups were determined using Fisher's exact test (p=0.01).

Secreted immune mediators in cervicovaginal lavages

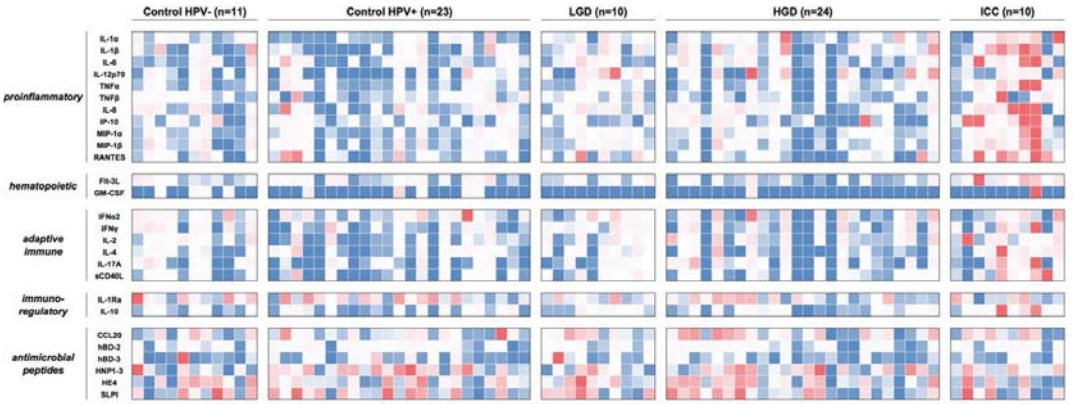

Figure 4. Patients with ICC, but not LGD or HGD exhibit increased levels of proinflammatory cytokines/chemokines relative to controls. Level of immune mediators in cervicovaginal lavages (CVL) was determined using Luminex analysis. Heat map reflects relative levels of immune mediators across all the samples. Increasing brightness of red and blue indicate higher and lower concentrations of each protein, respectively.

Associations between immune mediators, cancer and demographic variables

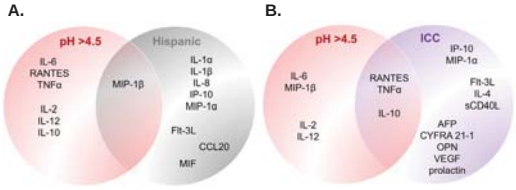

Figure 5. Unique immune signatures are associated with pH, ethnicity and cancer. The Venn diagram presenting immune mediators in CVLs significantly associated with pH >4.5 and ethnicity **(A)** or pH >4.5 and invasive cervical carcinoma (ICC) **(B)**. Association between level of immune mediators and demographic variables was performed in all collected samples using linear mixed effect model. The differences in the levels of immune mediators between groups were also tested using linear mixed model.

Increased levels of immune mediators in ICC patients

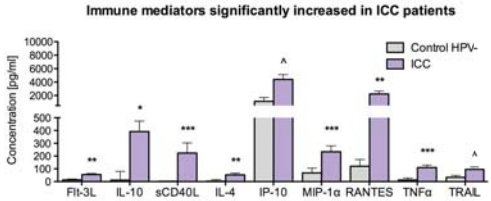

Figure 6. Patient with ICC exhibit significantly increased levels of proinflammatory and regulatory cytokines. Levels of immune mediator in cervicovaginal lavages (CVL) was determined using Luminex analysis. The difference in the concentration between the groups was tested using mixed linear model where group is fixed effect and replicate is the random effect. If the overall difference was significant (P<0.05), the paired test was performed with Bonferroni adjustment. ^ P<0.05; * P<0.01, ** P<0.001; *** P<0.001.

Cancer biomarkers in cervicovaginal lavages

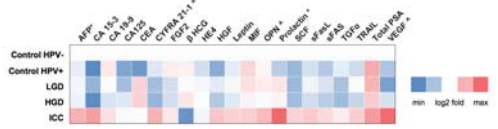

Figure 7. Patients with ICC, but not LGD or HGD exhibit significantly increased levels of cancer biomarkers relative to controls. Level of immune mediators in cervicovaginal lavages (CVL) was determined using Luminex analysis. Heat map reflects $\log_2$ fold changes in average levels of cancer biomarkers within each group. Increasing brightness of red and blue indicate increase or decrease of cancer biomarkers levels, respectively, compared to HPV-negative controls. The difference in the concentration between the groups was tested using ANOVA. ^ P<0.05; * P<0.01.

Conclusions

- Patients with ICC, but not LGD or HGD exhibited significantly increased levels of IP-10, MIP-1α, RANTES, TNFα, Flt-3L, IL-10, sCD40L, IL-4, AFP, CYFRA 21-1, OPN, VEGF and prolactin relative to HPV-negative controls
- Vaginal pH is associated with ethnicity and exhibits an increasing trend during cervical cancer progression.
- High vaginal pH (>4.5) and Hispanic ethnicity were significantly associated with higher levels of tested immune mediators
- This study revealed unique immune signatures in the microenvironment associated with cervical carcinogenesis and in our Hispanic population

Ongoing work

- CVL samples will be applied to our 3-D human vaginal epithelial cell model to determine host inflammatory signatures and barrier function
- Bacterial DNA was extracted from batched vaginal swabs and VMB communities will be defined by 16S rRNA sequencing and compared to immune mediators
- Overall this study may reveal unique host immune response signatures associated with specific VMB communities in the cervicovaginal environment that can be targeted to prevent development and/or progression of cervical cancer.

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