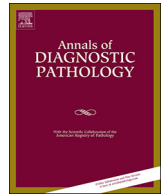




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Original Contribution

The distribution of novel biomarkers in carcinoma-in-situ, microinvasive, and squamous cell carcinoma of the uterine cervix

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ABSTRACT

Importin- β , exportin-5, p16, Ki-67, Mcl1, PDL1, and cFLIP are each over-expressed in the majority of CIN 1 lesions. These biomarkers, plus HPV E6/E7 RNA, were analyzed in carcinoma-in-situ (CIS), microinvasive, and squamous cell carcinoma (SCC) of the uterine cervix and cervical carcinoma cell lines. Only p16 and Ki-67 continued to be over-expressed in CIS, with a concomitant marked increase in E6/E7 RNA. There was a highly significant increase in PDL1 expression and decrease in Ki-67 (each $p < 0.001$) in microinvasive cancer compared to CIS whereas p16 and E6/E7 remained stable. As the lesion progressed to SCC, p16 and E6/E7 RNA remained strongly overexpressed with a concomitant over expression of importin- β and Ki67. HPV positive Caski cells showed significant elevations of p16, importin- β , exportin-5 and PDL1 compared to the HPV negative cervical cancer cell line C33A, consistent with viral induction of these biomarkers. The data suggest that PDL1 may be a useful biomarker to differentiate CIS from microinvasive cancer and, thus, anti-PDL1 therapy may inhibit the progression of CIS to the invasive stage.

1. Introduction

Uterine cervical cancer is the fourth most frequent cancer in women worldwide, representing 7.9% of all female cancers and accounting for 7.5% of all female cancer deaths, although in some countries it is the most common cause of cancer death in women [1,2]. The incidence of cervical cancer can be reduced through screening programs, followed by early diagnosis and treatment [2], and through the HPV vaccine that is highly effective if administered prior to infection [3]. Nonetheless, cervical cancer will remain a serious health issue in unvaccinated women including in the United States where the incidence has not decreased since the onset of HPV vaccination with African-American and Hispanic women at much greater risk [4].

The use of targeted approaches has recently improved clinical outcomes in a variety of cancers. One example is the use of checkpoint inhibitors that can reduce tumor size in about 25% of patients with a given non-resectable cancer who have mounted a strong T cell response which is blocked by PD1 or PDL1 [5]. The discovery of novel targeted

therapeutics requires a comprehensive understanding of the pathogenesis of the disease which, in turn, is facilitated by in situ analyses as this allows the precise localization of a given target to dysplastic cells as they progress to cancer and the concomitant host response [6]. Using this approach, PDL1 expression was shown to be significantly greater in cervical SCC compared to other gynecologic malignancies [7]. This, in turn, may reflect the fact that of these malignancies only cervical cancer is strongly correlated to HPV infection since viral infection per se can increase PDL1 expression [8].

Acute viral infection in general, and HPV infection in particular, induces a broad-spectrum host response that involves major pathways such as toll like receptors and NFK- β [9,10]. In this regard we recently described several downstream proteins of these two pathways that include importin- β , exportin-5, c-FLIP, Mcl1 and PDL1 as well as the standard biomarkers Ki-67 and p16 that were markedly increased in CIN 1 (CIN 1) [9]. Interestingly, each of these biomarkers strongly localized to the dysplastic cells in the parabasal zone marked by relatively quiescent low copy HPV DNA [9].

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Importin- β and exportin-5 are key proteins in the transport of RNAs and proteins into and out of the nucleus. Cells often use such nuclear trafficking to repress or activate constitutively expressed proteins such as p53 and NF κ B [11]. Nuclear trafficking is important in HPV infection. The HPV E6 protein cannot degrade p53 if exportin-5 mediated nuclear export of p53 is blocked [12]. Further, the apoptosis-inhibitory protein cFLIP is up regulated by viruses and malignant cells to abrogate cell death as does Mcl1 that suppresses BAK/BAX-induced apoptosis [13–15].

The screening for and treatment of cervical precancers costs about 8 billion dollars/year in the United States and yet CIN (synonymous with squamous intraepithelial lesion, SIL) remains the most common viral sexually transmitted disease [1,2]. Common problems including misdiagnoses of CIN 1–3 and microinvasive disease underscore the need for new biomarkers for more accurate diagnosis. The purpose of this paper was to determine if the novel biomarkers recently described in CIN 1 [9] are dysregulated in CIS, microinvasive and deeply invasive SCC and, thus, determine if any of these biomarkers can assist in the diagnosis of microinvasive cancer or demonstrate an increased risk for disease progression.

2. Materials and methods

2.1. Specimens, study design and study population

A cross sectional study that included tissue microarray (TMA) slides of formalin-fixed paraffin embedded human squamous cell carcinoma (SCC) were obtained from US Biomax (TMA CR1501). Clinical information included the women's age, stage, and grade of the cancer. Additionally, ten cases that included LEEP cervical excisions diagnosed as microinvasive SCC obtained from the National Institute of Health of Women, Children, and Adolescents, Fernandes Figueira – IFF-FIOCRUZ in Rio de Janeiro, Brazil as per the respective IRB protocol were studied. Two gynecologic pathologists (GJN and CVA) evaluated the cores and LEEPs and arrived at consensus diagnoses for CIN 3/high grade SIL and microinvasive/deeply invasive SCC as well as evaluating the immunohistochemistry. Negative controls for the immunohistochemistry included ten cervical tissues with unremarkable histopathology that were taken adjacent to a SCC. Ten CIN 1 cases served as positive controls for the immunohistochemistry testing of the various biomarkers.

2.2. In situ hybridization

HPV 16/18 E6/E7 RNA was detected by in situ hybridization using the RNAscope kit (ACD, Newark CA) as well as the Enzo HPV E6/E7 RNA kit (Enzo Life Sciences, Farmingdale, NY) per the manufacturer's protocol as previously published [16]. Likewise, HPV DNA was detected using a previously published protocol and the full length biotin tagged genomic probes from Enzo [2,16].

2.3. Immunohistochemistry assay

The immunohistochemistry protocol was carried out as previously published [17,18]. Cervical specimens were tested for the following antigens: cFLIP, importin- β , exportin-5, p16, PDL1 (28-8 clone), Ki-67 (ABCAM, Cambridge, MA), Mcl1 (Enzo Life Sciences, Farmingdale, NY) and HPV consensus L1 capsid protein (BIOCARE Medical, Pacheco, CA). Testing was performed on the automated Leica Bond platform as previously reported [16–18] which included pretreatment for 40 min with the antigen retrieval 2 solution with the modification that the Enzo Life Sciences peroxidase anti-mouse/rabbit conjugate (catalogue # ADI-950-113-0100) was used in place of the equivalent Leica conjugate as this reduced background [18].

2.4. Quantification of immunostained cells

Quantification of the immunostained cells was done in an automated fashion by the InForm system (Perkin-Elmer) and where indicated manually by two gynecologic pathologists (GJN and CVA). The computer-based InForm system can quantify the percentage of cells of a given type positive for the target of interest. The cores were categorized as follow: (0), positivity in < 1% of the neoplastic cells; (1+), positivity higher than 1% and < 10%; (2+), 10% to < 30%; (3+), 30% or higher.

2.5. Organotypic epithelial raft culture

Existing tissue from HPV16 positive Caski cell organotypic cancer rafts was utilized as previously described [19–22]. A total of 1×10^6 cells was plated on a collagen matrix that harbored embedded fibroblasts as a dermal equivalent. Exposure to the liquid-air interface resulted in the generation of 3D cancer as a model of CIS. Rafts were harvested 14 days after lifting, fixed in 4% paraformaldehyde, and embedded in paraffin. HPV16 positive Caski and HPV negative C33A cervical cancer cells were cultured as previously described [10].

2.6. Statistical analysis

Descriptive statistics of the qualitative variables was determined by frequency distribution and quantitative variables by medians and interquartile ranges (IQR) or mean and standard deviation (SD). Afterwards, the Chi-square test was used for categorical variables and Mann-Whitney *U* test for continuous variables. The Spearman correlation test was used to analyze possible associations among the biomarkers expression and histopathological grading of lesions both at 95% confidence intervals (CIs) and *p* value ≤ 0.05 . The agreement rate between the InForm and the pathologist counting the stained cells was performed using the Kappa test with 95% CI and *p* value ≤ 0.05 .

3. Results

3.1. Marked overexpression of select biomarkers in SCC

A cervical cancer TMA was analyzed that included 60 SCC and normal adjacent cervical tissues. The women's age distribution was between 30 and 39 (10%), 40–49 (43.3%), and 50 years or older (46.7%). Most tumors were grade 2 (60.4%) or grade 3 (16.0%) and stage I (80.0%) or stage II (18.3%). Each of the four putative biomarkers (importin- β , exportin-5, Mcl1, cFLIP), and also p16, and Ki-67, was scored in a blinded fashion. The PDL1 data for the cervical SCC was published previously by our group; 51% of cervical SCC tissue showed a strong signal in the invasive cancer cells for PDL1 [23].

Quantitative analysis of the immunohistochemistry data was initially done by manual pathological analysis and the computer based InForm system for the SCC cores. The proliferation marker Ki-67 was used to compare these two different methods of quantification, and excellent agreement was found with a concordance score of Kappa = 0.91. Thus, subsequent quantification of markers for SCC was derived by manual counts of the percentage of signal positive cancer cells. The combination of samples with intermediate (2+) and strong (3+) expression was defined as marked overexpression of a given marker (Table 1). Normal adjacent cervical epithelia cores in the TMA showed no signal for importin- β , Mcl1, p16, or cFLIP (data not shown). Benign cervical squamous epithelia did show a signal for Ki-67 and exportin-5, but this was restricted to scattered cells in the basal zone.

Note from Table 1 that the proteins with marked overexpression in SCC were importin- β (91.8%), p16 (90.6%) and Ki-67 (82.8%). Exportin-5 (46%), Mcl1 (46%) and cFLIP (4%) showed less expression compared to the other three markers (*p* < 0.001). Representative images for the SCCs are provided in Fig. 1; note the strong expression of

Table 1
Expression of importin- β , exportin-5, p16, cFLIP, Ki-67, and MCL-1 in cervical SCC.^{a,b}

Score	Importin- β % of cases	Exportin-5 5% of cases	P16% of cases	cFLIP % of cases	MCL1% of cases	Ki67% of cases
0	3.6	49.1	6.0	76.7	24.3	1.7
1 + ^a	4.5	11.7	3.4	18.9	27.8	15.5
2+	12.7	11.0	4.3	3.4	25.2	23.3
3+	79.2	28.2	86.3	0.9	22.6	59.5

^a 1+ higher than 1% and < 10% of cancer cells +; 2+ from 10% to 29%+; 3+ 30%–100%+.

^b Strong PDL1 expression was evident in 51% of cervical SCC (carcinoma cells) as previously reported [7].

exportin-5 in the parabasal zone of the CIN 1 that is marked by low HPV copy number, and a lack of overexpression of exportin-5 in SCC with high expression of p16, Ki67, and importin- β and with a uniform HPV DNA in situ pattern.

Next, we analyzed the extent to which the biomarker data correlated with Ki-67 (proliferative index), patient age, or stage/grade of the disease. There was no correlation between the biomarkers scores and age ($p = 0.775$). Mcl1 ($p = 0.018$) and Ki-67 ($p = 0.015$) had a significant correlation, albeit weak, with tumor grade, where each protein tended to be more strongly expressed at higher grades. Tumor stage showed an inverse correlation with p16 ($p = 0.001$) and Mcl1 ($p = 0.002$). Importin- β ($p < 0.001$), exportin-5 ($p = 0.01$) and p16 ($p = 0.001$) each correlated strongly with Ki-67 expression.

3.2. PDL1 is a highly selective marker of microinvasive SCC versus CIS

Ten LEEP cervical biopsies that contained microinvasive SCC were available for study. The microinvasive component in each case was < 3 mm in depth and < 5 mm in lateral extension with no definitive area of lymphovascular invasion and the margins were clear. In each case there was extensive CIN 3 that is referred to as carcinoma-in-situ (CIS) when it was located within 5 mm of microinvasive disease. Otherwise, the term CIN 3 is used for the high-grade dysplasia that was > 5 mm away from an invasive nest. It should be stressed that we are not advocating the use of the term CIS but, rather, simply comparing high grade CIN/SIL direct adjacent to invasive cancer (CIS) to the same histology located at least 5 mm away from the invasive foci. Quantification of each biomarker was done with the InForm computer based system and in each case CIS versus microinvasive SCC was scored from the same 200 \times field.

There was no difference in the expression of any of the biomarkers when comparing CIS to CIN 3 in the microinvasive cases (data not shown). Table 2 presents the comparison of the biomarkers in CIS versus microinvasive cancer. The most striking difference was the highly significant increase in PDL1 expression in microinvasive cancer versus CIS. This is illustrated in Fig. 2. We also note the near loss of PDL1 in CIN 3/CIS which means that this protein is down regulated as the lesion progresses from CIN 1 since PDL1 is strongly expressed in the parabasal cells in the latter [9]. Next, HPV 16/18 E6/E7 RNA were highly expressed in CIS and CIN 3 whereas, as reported previously, these oncogenic viral RNAs are rarely detectable in CIN 1 that harbors either of these HPV types [9]. Finally, using the stringent criterion of $p < 0.001$ when comparing CIS to microinvasive SCC, then beyond PDL1 overexpression, only the Ki-67 index was significantly different in microinvasive SCC versus CIS, although it was much decreased in the microinvasive component.

3.3. Caski cells versus C33A cells demonstrates HPV increases biomarker expression

Caski cells are HPV 16 positive cells whereas C33A are HPV

negative cells, each derived from cervical cancers. We studied the effect of HPV infection on the expression of the different biomarkers. The data is presented in Table 3. There was a > 4 fold increase in the expression of p16, importin- β , exportin-5, and PDL1 in the Caski cells when compared to the C33A cells. Next, the expression of the different biomarkers was compared in Caski cells grown in organotypic cancer rafts. As summarized in Table 3, there was an equivalent percentage of Caski cells expressing HPV 16 E6/E7 RNA, p16, and Ki-67 in 3D organotypic rafts versus conventional 2D culture. However, there was a marked reduction in PDL1 expression and a marked increase in importin- β , Mcl1, and exportin-5 expression in the cells grown in the raft versus conventional culture (Fig. 3). Thus, the 3D growth of Caski cells in differentiating conditions led to reduced PDL1 expression and increased nuclear trafficking and Mcl1 expression.

Representative images of the C33A and Caski cells (cell culture and organotypic raft) are presented in Fig. 3. Detection with the ACD and Enzo Life Sciences E6/E7 probes is strong and equivalent in both cell lines and in 3D rafts. An advantage of the latter assay is that one step (the AP conjugate) is required for detection whereas six separate steps are required for detection with the ACD RNAscope assay.

4. Discussion

Precursor lesions that are prerequisite for the development of cervical cancer may be the best characterized among human carcinomas. CIN 1/low grade SIL, which represents acute HPV infection, evolves in about 30% of cases to high grade CIN (CIN 2/3)/SIL that is the obligatory precursor to the invasive lesion [2]. CIN 3 is now rarely referred to as carcinoma-in-situ (CIS) although, in this study, the term CIS was restricted to CIN 3 in the immediate vicinity of microinvasive cancer strictly for statistical analyses. CIS initially proceeds to microinvasive cancer where cure rates of nearly 100% are anticipated after surgical removal of the lesion. This compares to a 50% five-year survival in stage II cervical cancer and a 15% five year survival in stage IV cancer [2]. Since CIN is a sexually transmitted disease, much attention has been focused on reducing false positive diagnoses as well as being able to better predict which lesions have a higher likelihood of progressing to invasive cancer [2,24,25]. In this paper we present molecular data that assist in clarifying these questions by underscoring the following: 1) CIN 1 is marked by the expression of multiple proteins that are present in the parabasal zone of the lesion that co-express with low copy, relatively quiescent viral DNA [9]; 2) progression to CIN 3 leads to the loss of some of these biomarkers (importin- β , exportin-5, Mcl1, cFLIP, PDL1) and the continued expression of p16 and Ki-67 with the marked up regulation of the viral oncoproteins E6 and E7; 3) CIS and CIN 3 are, as expected, molecularly indistinguishable relative to these biomarkers; 4) microinvasive SCC can be differentiated on a molecular basis from CIS by marked increased PDL1 and decreased Ki-67; 5) as the lesion progresses to deeply invasive SCC, Ki-67 and importin- β are upregulated and PDL1 decreases in the cancer cells but increases in the infiltrating inflammatory cells. These observations are graphically illustrated in Fig. 4.

Importin- β is a member of the karyopherin superfamily that comprises several nuclear transport proteins. The overexpression of importin- β is derived from deregulated E2F activity due to HPV E7 repression of retinoblastoma protein (Rb) [11]. This may explain in part why E6/E7 and importin- β were strongly and diffusely expressed in SCC of the uterine cervix. However, there might be a different explanation for the abundant expression of importin- β in CIN 1 lesions since E6/E7 RNA levels were very low in such tissues [9]. The complexity of importin- β expression in cervical oncogenesis was underscored by its high expression in CIN 1, marked reduction in CIN3/CIS and microinvasion, and marked increased expression in deeply invasive SCC.

p16 is well documented to be a biomarker of E6/E7 RNA expression since the inhibition of the p53/Rb pathways can lead to a compensatory

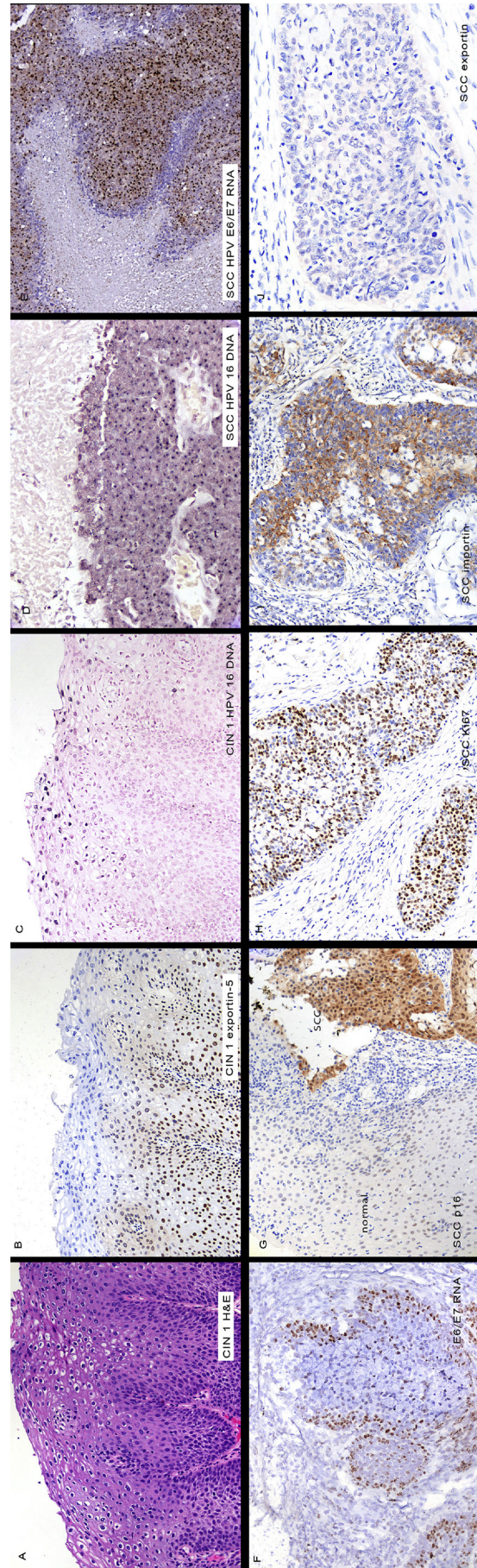


Fig. 1. Biomarker expression in SCC of the cervix versus CIN 1. Panel A: H&E of CIN 1 used as positive control for SCC study. Panel B: Exportin-5 is strongly expressed in the lower 2/3 of the CIN 1 lesion whereas HPV 16 DNA is most abundant in the upper 1/3 (panel C). Panel D: HPV 16 DNA shows the typical punctate pattern in this SCC whereas HPV 16 E6/E7 RNA is likewise detectable in most of the cancer cells in the serial section (panel E). Panel F: Another common pattern for HPV 16/18 E6/E7 RNA was the localization of the positive cells towards the periphery of a given cancer nest whereas p16 showed a signal in each cancer cell (Panel G; note the adjacent normal epithelia which served as an internal negative control). The next three panels are serial sections of an SCC which show a strong signal in most cancer cells for Ki-67 (H), importin-β (Panel I) and no signal for exportin-5 (panel J).

Table 2
Comparative expression of importin-β, exportin-5, p16, Ki-67, and MCL-1 in CIS versus microinvasive SCC.^a

Category	Importin-β	Exportin-5	P16	PDL1	MCL1	Ki67
CIS	1074/4521 (23.7%)	1100/2314 (47.5%)	5638/6435 (87.6%)	329/5066 (6.1%)	1146/3299 (34.7%)	2319/4032 (57.5%)
Microinvasive SCC	1034/3249 (31.8%)	541/1725 (31.3%)	4022/4409 (91.2%)	1630/3492 (47.5%)	1045/1920 (54.4%)	918/2729 (33.6%)
Chi-square statistic/p value	2.03/0.154 NS ^b	5.95/0.015 NS	0.48/0.4888 NS	43.15/ < 0.001 SIGN	7.31/0.007 NS	10.66/0.001 SIGN

^a cFLIP quantification was not done as it was low in each case and also, due to the many sections required for analysis with controls, several of the cases lacked the microinvasive component in the deeper sections for cFLIP which was the last protein studied.

^b NS = no significant difference and SIGN = significant difference.

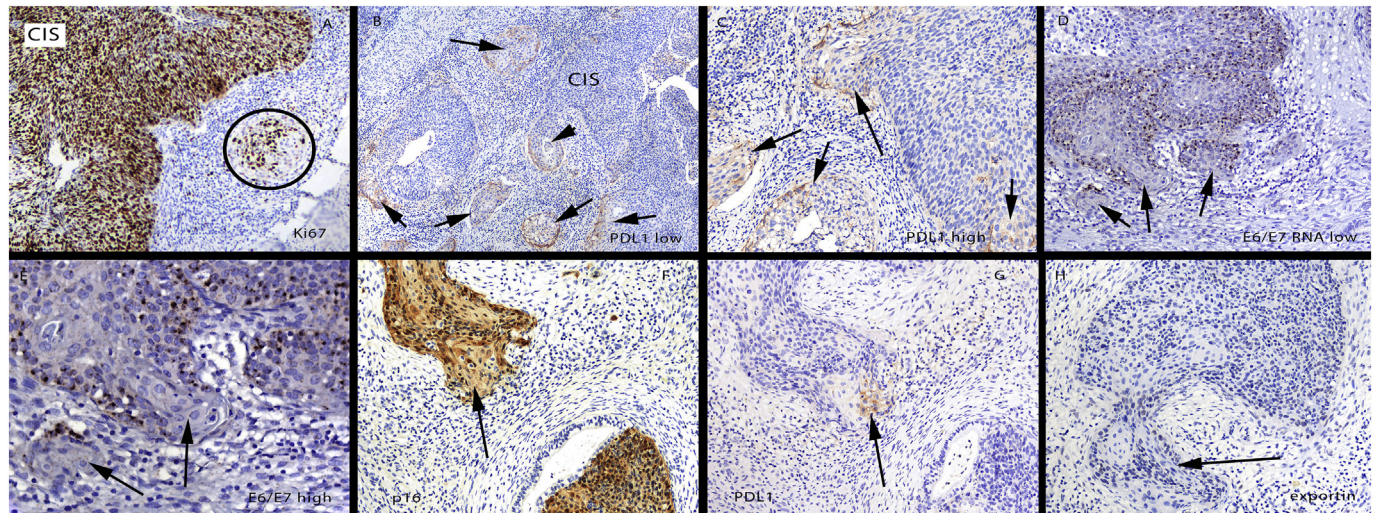


Fig. 2. Biomarker expression in microinvasive SCC of the cervix. Panel A: Ki-67 protein shows a strong signal in > 90% of the CIS cells and in a smaller percentage of cells in the nest of microinvasive SCC (circle). Panels B (low magnification) and C (high magnification) show an area of CIS with several foci of microinvasive SCC in which PDL1 is not expressed in the CIS but is strongly expressed in the microinvasive cancer cells (arrows). Panels D (low magnification) and E (high magnification) show that HPV 16 E6/E7 RNA is expressed in both the CIS and microinvasive cancer cells, although it rarely is found in the microinvasive cancer cells with “paradoxical differentiation”. Panels F, G, and H are serial sections of an area of CIS extending into a gland and adjacent microinvasive SCC (arrows) where p16 is strongly expressed in both areas (F), PDL1 localizes to only the microinvasive cancer cells (G) and exportin-5 is not evident in either area.

Table 3
Tabulation of data for Caski vs C33A cervical cancer cells.

Cell line	HPV E6/E7 RNA ^a	HPV DNA	Ki-67	Importin-β	Exportin-5	PDL1	MCL1	p16
C33A	0	0	79.3% (2.5)	0.7% (0.2)	3.9% (0.9)	0	49.2% (6.1)	6.9% (1.3)
Caski cell culture	54.2% (4.5)	100%	89.8% (1.9)	7.9% (1.5)	16.5% (4.7)	82.1% (6.3)	56.9% (7.4)	95.9% (1.3)
Caski raft	51.0% (6.0)	100%	96.3% (3.3)	81.8% (3.9)	93.9% (2.8)	13.3% (1.9)	93.1% (4.5)	94.1% (2.3)

^a Each data point is the mean % (SD) for a minimum of 850 cells.

p16 up regulation [2,20-22]. Given the strong E6/E7 expression in SCC and CIS it is not surprising that p16 is also much increased. However, a different mechanism must be operative in CIN 1. In this regard it is important to stress that, as previously documented, commercially available HPV E6/E7 RNA probes can cross react with viral DNA and, thus given a false signal in cases where it can be documented that viral E6/E7 RNA is not present [9]. Other biomarkers highly expressed in CIN 1 that were not evident in CIN 3/CIS or microinvasive/deeply invasive cancer included cFLIP, Mcl1, and exportin-5. Although additional work is needed to understand the molecular basis for their loss of expression in cervical oncogenesis, it is clear that none of these proteins are good biomarkers for tracking possible progression of early CIN.

The HPV positive Caski cells showed much stronger expression of importin-β, exportin-5, p16, and PDL1, though not Mcl1, when compared to the HPV negative C33A cervical cancer cells grown in culture. These observations underscore the fact that viral infection in general and HPV in particular induces the expression of many proteins including nuclear transport proteins, PDL1, and various other proteins that can assist the cells in avoiding immune surveillance, apoptosis, or

senescence [8,9].

The histologic diagnosis of microinvasive SCC of the cervix can be difficult. High grade CIN often extends into endocervical glands that can mimic microinvasive disease. Immunohistochemistry stains for basement membrane disruption that include type IV collagen, and laminin as well as a reticulin staining may have benefits in identifying microinvasion although tangential sectioning can hinder their interpretation [2]. Histologic features such as irregular shaped nests of dysplastic cells and paradoxical squamous cell “maturation” also are useful in diagnosing microinvasive cancer and, thus, differentiating from CIN3 [2]. Although useful, none of these variables address molecularly changes that occur at the initiation of invasion in CIS lesions. A surprising result in this study was that expression of PDL1 was a reliable and highly significant marker of early invasion when compared to CIS; to our knowledge, this has not been documented previously. It should be emphasized that the PDL1 expression analysis was limited to the cancer cells, and not the surrounding lymphocytes. Indeed, it was evident that PDL1 expression in microinvasive SCC of the cervix was predominantly from the invasive squamous cells as compared to deeply

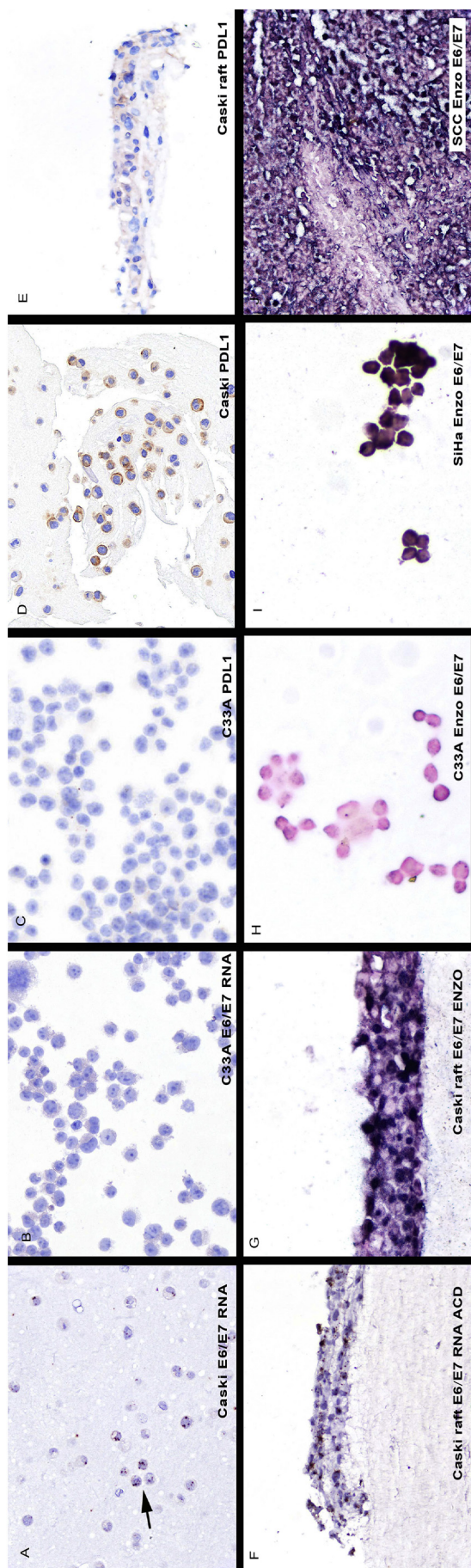
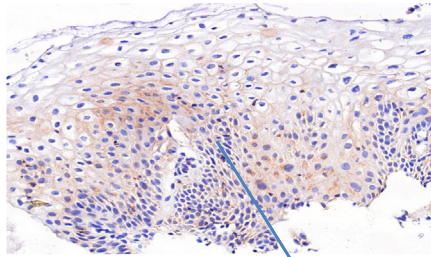


Fig. 3. Biomarker expression cervical cancer cell lines: C33A and Caski including organotypic raft versus cell culture. Panel A: HPV 16 E6/E7 RNA is found in many Caski cells grown in culture with the ACD assay (arrow) but not in the HPV negative cervical cancer cell line C33A (panel B). Panels C-E: PDL1 expression was not evident in the C33A cells grown in culture (panel D). However, the PDL1 expression was much reduced in the Caski cells when grown in the raft assay (panel E). Panels F-J: HPV E6/E7 was intense in the Caski cells grown in the organotypic raft with the ACD assay (panel F) and the Enzo Life Sciences assay (panel G). No signal was evident in the C33A cells with the Enzo E6/E7 probe (panel H) whereas an intense signal was seen in the SIHa cells that contain 1 copy of HPV 16 DNA (panel I). Panel J shows the intense signal with the Enzo E6/E7 probe in a cervical SCC which is the same cancer shown in Fig. 1E which depicts the ACD assay.

CIN 1: High p16, Ki-67, importin- β , exportin-5, MCL1, cFLIP, PDL1 towards base of lesion



Basal aspect

CIN 3/CIS: High p16, Ki-67, E6/E7; Loss of importin- β , exportin-5, MCL1, cFLIP, PDL1

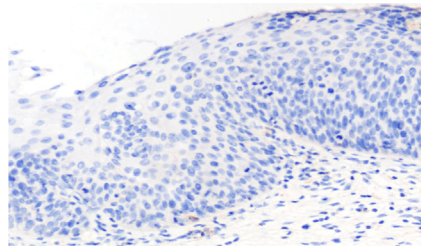
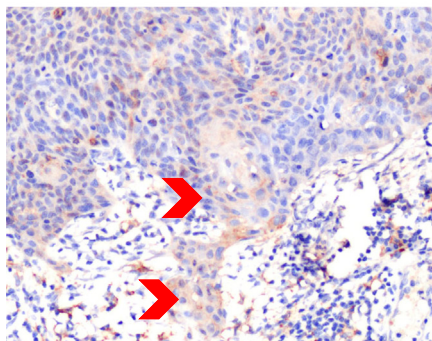


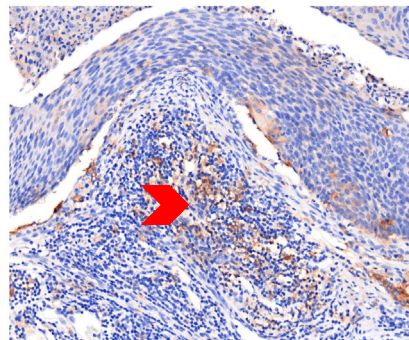
Fig. 4. Graphic representation of the molecular changes from CIN 1 to invasive SCC. In CIN 1, multiple biomarkers including MCL1, cFLIP, importin- β , exportin-5, PDL1, Ki67 and p16 are over-expressed in the cells with low copy relatively quiescent HPV DNA that localize to the base of the lesion (PDL1 depicted). In the cases where the CIN progresses to CIN3/CIS, only p16, Ki-67, and E6/E7 are over expressed; the image depicts the loss of PDL1 in a CIS lesion. If the lesion progresses to microinvasive cancer, then PDL1 expression is markedly increased (arrows depict the paradoxical differentiation); the image is the microinvasive cancer directly adjacent to the CIS in the prior panel. In cases where the lesion becomes deeply invasive, PDL1 is strongly expressed but primarily in the intratumor mononuclear cells (arrow) whereas p16 and E6/E7 expression remain stable and there is an increase in importin- β and Ki-67.

PDL1 expression shown in each image

Microinvasive SCC: increased PDL1, decreased Ki67, stable p16, E6/E7



SCC: increased Ki67, importin- β , stable p16, E6/E7, PDL1



invasive SCC of the cervix where mononuclear inflammatory cells, including CD8 cells and macrophages, were the primary source of PDL1 [9]. This data suggests that an important molecular switch during evolution of CIS to microinvasion is the acquisition of PDL1 expression and that in time, as the tumor becomes deeply invasive, an immune response may be mounted against the tumor where PDL1 expression shifts to the immune cells. Other studies have underscored the importance of PDL1 expression in cervical oncogenesis as well as in HPV positive head and neck cancers [5,7,26].

Another interesting molecular switch from CIS to microinvasion noted in this study was the significant reduction of Ki-67 expression. Although the molecular reason for this result will require further study, it may be related to the paradoxical “maturation” of the dysplastic squamous cells in microinvasion since such cells typically are not mitotically active [2].

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Disclosure

The authors declare no conflict of interest.

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