CD83 Gene Polymorphisms Increase Susceptibility to Human Invasive Cervical Cancer

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Abstract
We previously mapped a nonrandom frequent loss of heterozygosity (LOH) region in cervical cancers to 1 Mb of 6p23. Here, we describe the identification of a novel cervical cancer susceptibility gene, CD83. The gene was identified by several complementary approaches, including a family-based association study, comparison of transcript expression in normal and cancerous tissue, and genomic sequencing of candidate. CD83 encodes an inducible glycoprotein in the immunoglobulin superfamily and is a marker for mature dendritic cells. The association study that includes 377 family trios showed that five single nucleotide polymorphisms (SNP) within 8 kb of its 3′-end showed significant allelic association that was strengthened in a subgroup of women with invasive cancers infected by high-risk human papillomavirus type 16 and 18 (rs9296925, P = 0.0193; rs853360, P = 0.0035; rs9230, P = 0.0011; rs9370729, P = 0.0012; rs750749, P = 0.0133). Investigation of CD83 uncovered three alternative transcripts in cervical tissue and cell lines, with variant 3 (lacking exons 3 and 4) being more frequent in cervical cancer than in normal cervical epithelium (P = 0.0181). Genomic sequencing on 36 paired normal and cervical tumors revealed several somatic mutations and novel SNPs in the promoter, exons, and introns of CD83. LOH was confirmed in >90% of cervical cancer specimens. Immunofluorescence colocalized CD83 protein to the Golgi apparatus and cell membrane of cervical cancer cell lines. None of seven nearby genes was differentially expressed in cervical cancer. The importance of CD83 in epithelial versus dendritic cells needs to be determined, as does its role in promoting cervical cancer. [Cancer Res 2007;67(23):11202–8]

Introduction
Human papillomavirus (HPV) infection is necessary for the development of cervical cancer. However, it is not sufficient by itself because an estimated 30% to 60% of sexually active women are infected with genital HPV yet do not develop cancer (1). The 10-year cumulative incidence rates of immediate cancer precursor cervical intraepithelial neoplasia 3 (CIN3) or invasive cervical cancer (ICC) are 17.2% for women infected with HPV16 and 13.6% for those infected with HPV18 (2). Furthermore, the transition from CIN to invasive cancer has a long latency period, and only a minority of those infected go on to develop ICC. Moreover, a large proportion of women clear HPV infection (3). Therefore, identifying nonviral factors that permit the virus to cause cancer is important for understanding viral-associated malignancies.

Compelling evidence has implicated genetic factors in susceptibility to CIN3 and ICC. Using the Swedish national registers, Magnusson et al. found a significant familial clustering of the disease. The consistent pattern of decreasing familial relative risk with decreasing degree of genetic relationship pointed to genes as the major cause of familial aggregation of cervical cancer (4). Of the several susceptibility markers identified, the association of HLA alleles with increased risk of CIN3 or ICC is most often reported (5, 6). A recent report of genomic mutations in the genes TMC6 and TMCS8 in patients with epidermodysplasia verruciformis, a rare dermatosis associated with a high risk of HPV5-related skin cancer, highlights the importance of genetics in progressive viral-associated cancers (7).

Candidate genes for carcinogenesis can be identified in areas of nonrandom chromosomal aberrations. We and others have identified distinct chromosomal alterations on chromosomes 3p, 6p, and 11q linked to cervical carcinogenesis using methods such as loss of heterozygosity (LOH), comparative genomic hybridization, and cDNA expression microarrays (8–12). Loss of 6p23 is an early event, which is detected in CIN (10, 13).

To identify causative candidate genes within 6p23, we first identified new protein-coding segments within this region by comparative analysis of human and mouse genomic sequence. Then, we evaluated seven known genes and nine unknown genes with at least one splicing event within and around this region. Finally, we conducted an association study using tag single nucleotide polymorphisms (SNP; ref. 14) to investigate whether germ-line sequence variants associate with susceptibility to ICC and CIN3.

Materials and Methods
Choice of candidate genes and expressed sequence tags for expression studies. In previous work, we detected a small (1 Mb) deletion...
on 6p23 of ICC patients between markers D6S429 and D6S1578 (13). This region has only one known gene, CD83, which lies at its telomeric end. We started looking for protein-coding segments in 6p23 by comparing it with the mouse sequence. Because this work began before the mouse genome was completely sequenced, we used the RPCI-23 mouse library to develop a bacterial artificial chromosome contig centered on CD83. Cold Spring Harbor Sequencing Center sequenced three overlapping clones covering 650,000 bp of that region. We then determined the homology of the mouse DNA with human DNA using PipMaker,10 which was developed by Dr. W. Miller’s laboratory at Pennsylvania State University. Percent identity plots (Pips) provide complementary information to GenScan and highlight database hits for finding exons and candidate regulatory elements.

We selected CD83 and nine expressed sequence tags (EST) centromeric to that gene within the LOH region to explore expression patterns in a panel of human cervical tissues and other tissue types. To further investigate potential candidate genes around the critical region, we expanded the survey region to 5 Mb from D6S470 to D6S1578. The expression of six more genes [PAK1/P1, NEDD9, SRT5, RANBP9, nuclear protein 7 (NOL7), and RNF12] telomeric to CD83 and with relevant biological functions was evaluated in the same panel of tissues.

**Subjects for the association study.** We obtained informed consent from 377 family trios. Each trio consisted of a proband—a woman with ICC (255) or CIN3 (122, CIN3 and/or adenocarcinoma in situ)—and either her biological parents or one parent and one or more siblings. Three hundred and forty-one trios were Caucasian, and 36 were African-American. Blood or buccal samples were obtained from all the subjects. Invasive cancers or CIN3 lesions from the probands were obtained for HPV typing. Among the 255 ICC, 167 tumors were squamous, 62 were adenocarcinoma, 9 were adenosquamous, and 17 were of other histologies. Of the 303 cervical tissues typed for HPV, 259 tissues are classified as HPV16 or HPV18 related. The study was approved by the Washington University’s Human Studies Committee.

**Cervical tissues and cell lines.** Frozen specimens included 8 normal cervical biopsies, 11 normal cervical epithelia, 14 ICC, and cancers and matching nonmalignant tissues from lung, liver, colon, spleen, prostate, kidney, breast, brain, thyroid, muscle, and lymph node. The normal cervical biopsies in our panel are enriched for the stratified epithelium near the transformation zone, where carcinogenesis is thought to begin. RNA from the epithelium of fresh-frozen normal cervix and ICC was microdissected by laser capture (PixCell IIe Laser Capture Microdissection System). The cell lines included two HPV16 E6/E7-transformed cervical epithelia cell lines, 14 cervical cancer–derived cell lines, 3 ovarian cancer cell lines, and 3 prostate cancer cell lines. The following cell lines were purchased from the American Type Culture Collection: CRL2614, CRL2615, HeLa, SiHa, CaSk, C41, HT3, ME180, C33A, SW756, DoTc2 4510, CRL2617, and MCF7. They were grown in the recommended medium.

**Identifying DNA polymorphisms and mutations in CD83 and NOL7.** CD83 spans 19 kb of genomic sequence and contains five exons. NOL7 spans 5,57 kb of genomic sequence and contains eight exons. To detect the most functional sequence variations, we designed primer pairs that were adequate distances from exon/intron boundaries. PCR products from CD83 were analyzed either by direct sequencing or by denaturing high-performance liquid chromatography (dHPLC) with the WAVE Nucleic Acid Fragment Analysis System (Transgenomic, Inc.). Those from NOL7 were analyzed by direct sequencing. The resulting sequences were compared with the corresponding reference gene sequences12 using DNAStar software (DNASTAR, Inc.). Thirty-six tumors and matched normal genomic DNA were evaluated for CD83 and 12 pairs for NOL7. Pooled genomic DNAs from the Caucasian and African-American populations were used to distinguish novel SNPs from mutations as described previously (16).

**SNP selection and genotyping.** Genomic DNA was isolated from blood by overnight digestion with proteinase K and extraction with phenol/chloroform and from buccal cells using Puregene DNA Purification kits (Gentra Systems, Inc.) using protocol provided by the manufacturer. The DNA was quantified by NanoDrop/ND-1000 Spectrophotometer and prepared at a concentration of 0.5 ng/μL and then loaded on 384-well PCR plates (3 μL/well) using the automated Apricot Personal Pipettor pp-150-MS (Perkin-Elmer).

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Because the 6p23 markers inLOH and microarray studies associate with both CIN3 and ICC (8, 13), we hypothesized that the locus may promote genetic susceptibility to the disease. To identify relevant genetic variants, we performed a region-based association study using family samples. Polymorphisms used for genotyping were either selected from the International HapMap Project database11 or identified by us during sequencing of candidate genes. For this study, we chose gene-based tag SNPs, defining haplotype blocks by a minimum r² of 0.8. The tag SNPs had to have a minor allele frequency of >5%. All putative functional mutations we identified were genotyped in the samples. The 24 tag SNPs span across 0.68 Mb from SRT5 to CD83. An additional 31 SNPs were added around RNF182 and CD83, close to markers with significant P value (P < 0.05). The genomic positions for all SNPs came from dbSNP build 127.

We performed genotyping with several methods. One, Taqman SNP Genotyping Assays (Applied Biosystems) using the primers and probe of the company. Two, pyrosequencing across the polymorphic sites (Biotech, Inc.) using primers designed and validated in house (available on request). The PCR profile and reaction conditions were tested and optimized using 24 individual control DNAs and pooled Caucasian or African-American DNAs. Genotyping success rates were >98% with the Taqman assays and >95% with pyrosequencing. Each genotyping plate contained water and DNA controls. The final genotypes were analyzed for transmission consistency between parents and offspring, and genotypes that showed Mendelian error were deleted and not used in analysis.

**RNA isolation, reverse transcription-PCR, and sequencing**

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**DNA Isolation and Microarray Analysis.** Genomic DNA was isolated from blood by phenol/chloroform and from buccal cells using Puregene DNA Purification kits (Gentra Systems, Inc.) using protocol provided by the manufacturer. The DNA was quantified by NanoDrop/ND-1000 Spectrophotometer and prepared at a concentration of 0.5 ng/μL and then loaded on 384-well PCR plates (3 μL/well) using the automated Apricot Personal Pipettor pp-150-MS (Perkin-Elmer).

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10 http://bio.cse.psu.edu

11 http://www.hapmap.org

a mouse monoclonal antibody to the human CD83 antigen; Invitrogen, Caltag Laboratories) or with the isotype control (mouse IgG1 purified; Invitrogen, Caltag Laboratories) for 1 h at room temperature in TBS containing 1% bovine serum albumin (BSA). After three TBS washes, the cells were reacted for 1 h with Alexa Fluor 488 goat anti-mouse antibody in TBS containing 1% BSA (50 μL/slide). For colocalization, cells were reacted with anti-human golgin-97 mouse monoclonal antibody (Invitrogen) for 1 h at room temperature and then with Alexa Fluor 594 goat anti-mouse antibody for 1 h. For nuclear staining, the same cells were treated with 4'-diamidino-2-phenylindole (DAPI; 1 μg/mL; Sigma) for 5 min. The stained cells were analyzed with a Nikon Eclipse TE2000-U inverted microscope using Qimage software (QImaging Corp.).

**HPV typing.** HPV typing was performed on tissue sections snap frozen in OCT or formalin-fixed, paraffin-embedded blocks modified from Li et al. (18). A HPV-negative control sample was cut between every tenth specimen and no cross-contamination was found in the HPV typing. DNA was extracted from the OCT blocks by the standard SDS-proteinase K procedure. It was extracted from the paraffin blocks using a Puregene DNA Purification kit. The DNA was amplified with primers to the conserved regions of HPV L1 (L1C1/L1C2M) and E6 (E6-L/E6-R). Aliquots of PCR were run on agarose gel and DHPLC and then sequenced. It was infected with multiple HPV types (indicated by multiple peaks on DHPLC), the PCR fragments were first separated by the fragment collector and then sequenced. Families were grouped according to the HPV type detected in the cervical neoplasia of the probands at diagnosis. HPV16-related types are HPV16, HPV31, and HPV52. HPV18-related types include HPV18 and HPV45.

**Association analysis.** We used the family-based test of association that is implemented in the program TRANSMIT (19) because it is robust to population stratification and our association study included trios of different ethnicities. A two-stage design was used to evaluate genetic variation in the LOH region. First, 24 SNPs were screened in a discovery set of 121 trios. SNPs significant at a nominal P = 0.05 were then typed in validation set of 134 trios. We reported the results of stage I discovery testing and our analysis of the combined stage I and II samples for optimal power (20). After having surveyed the 6p LOH region, SNPs within CD83 showed the strongest association; thus, we identified 31 additional tag SNPs located around CD83, assuming a loose block size (r² = 0.5). Five SNPs within CD83 showed marginal significance of the transmission/disequilibrium test of association. We examined haplotypes composed of these five SNPs, as well as all three SNP combinations, to determine whether these haplotypes showed stronger association than the individual SNPs. These tests can suggest whether the haplotypic background is important in tagging the functional variant. To identify possible heterogeneity of risk, we conducted follow-up tests by subdividing the sample into HPV16- and HPV18-related types, as supported by numerous studies (6, 21, 22).

**Results**

**Human and mouse DNA are highly homologous at 6p23.** We searched for homology between the human and mouse genome by studying ~ 650 kb of DNA centered on 6p23, first masking repeated sequences in the human DNA with the RepeatMasker program.13 The mouse sequence was obtained from British Columbia Genome Sequencing Center before the completion of the mouse genome sequence. The mouse sequence was aligned and compared with the human sequence using PippMaker. The protein-coding exons of many human and mouse genes show an average homology of ~ 85% (23, 24). We plotted percent identity (between 50% and 100%) of the sequences from the two species using coordinates of the human sequence, such as genes and repeats, along the horizontal axis. After plotting the homology of 17 predicted genes, we reached several important conclusions. First, CD83 showed strong homology at most of its five exons. Second, there was high homology (>75%) for the exons of several predicted genes of unknown function and this high level of homology provided the reason why the ESTs were evaluated for their expression.

**Expression of the candidate genes and EST in 6p23.** In total, seven known genes and nine ESTs within 6p23 were evaluated for expression by RT-PCR. PKH1P1, NEDD9 (NM 006403), SIRT5 variant 1, NOL7, and RANBP9 were not expressed differentially in normal or cancerous cervix. The short variant of NEDD9 (L43821) was expressed at a lower level in normal cervix than in ICC. The expression of CD83, SIRT5 variant 2, and RNF182 was higher in ICC or cervical cancer cell lines than in normal cervix. None of the nine ESTs that were centromeric to CD83 was expressed at significant levels in normal or cancerous tissue.

**SNPs in the 3' region of CD83 confer risk of ICC.** We analyzed 24 SNPs across 0.68 Mb on 6p23 in the LOH region that spans SIRT5 to CD83 in the discovery set of 121 families with ICC. An additional 31 SNPs were selected around CD83 for denser coverage where significant SNPs were identified at P < 0.05 in discovery set. Among the 55 SNPs analyzed, 7 were significant (P < 0.05). Along with a few adjacent SNPs, they were genotyped in the remaining 134 trios with ICC. The validation set comprised all 255 ICC trios and showed that five of the seven SNPs remained significant. The SNP association was strengthened when the families were subset by high-risk HPV types defined by probands with HPV16- and HPV18-related types (Table 1; Fig. 1). The five SNPs with significant P values were located within an 8-kb region of the 3'-end of CD83. However, these five SNPs were not significant in a smaller sample of CIN3 trios (Table 1). To eliminate the possibility that the CD83 SNPs conferred susceptibility to HPV16- and HPV18-related infections, we examined the distribution of the alleles in the five SNPs in women with HPV16- and HPV18-related cancers compared with other HPV types. We found no significant difference in the distribution of the alleles between groups. Haplotype analyses of these SNPs showed that a haplotype comprising markers rs9230- rs9370729-rs853362 was associated with ICC (0.001 < P < 0.009). The haplotype analysis did not add additional information to analysis of individual SNPs.

**Polymorphisms and mutations in CD83.** We identified polymorphisms in genomic DNA and also identified several somatic mutations in a panel of ICC and cell lines. First, one tumor contained a 1-bp deletion (174 C/→) 5 bp before the start codon ATG. Second, the cell line SW756 contained a two-nucleotide deletion (TT591-592/C0). This frameshift deletion creates a nonsense mutation, truncating the protein to 137 amino acids. Several novel SNPs were also identified in the promoter region and exons (Table 2). The polymorphisms were validated by methods other than sequencing, such as DHPLC. The DHPLC results and sequencing confirmed LOH in the region because among informative SNPs in CD83 21 of 23 ICC indicated that only one allele was preserved.

**Alternatively spliced CD83 mRNAs.** We identified three constitutively expressed transcripts of CD83 in the cervical cancer cells. The full-length five-exon transcript of 618 bp (CD83-1) and two splice variants were confirmed by direct sequencing. Variant 2 (CD83-2) lacked entire exon 3 and had only 389 bp. Variant 3 (CD83-3) lacked both exon 3 and exon 4 and had only 282 bp. The three alternatively spliced transcripts are predicted to encode three different proteins. CD83-1 would encode a protein of 205 amino acids and was expressed in all of the cell lines and human tissues.

13 http://repeatmasker.genome.washington.edu
tested, including cervical, ovarian, breast, and prostate cancers. CD83-2 (79 amino acids) was detected in 6 of 12 ICC cell lines and 5 of 8 ICC tumors. CD83-3 (93 amino acids) was seen in 4 of 8 ICC but in none of the 11 samples of normal cervical epithelium ($P = 0.0181$; Table 3; Fig. 2). This variant showed a similar differential expression trend between normal (4 of 31 positive, 12.9%) and cancerous (4 of 20 positive, 20%) of other tissue types.

**CD83 protein is localized to the cytoplasm of cervical cancer cells.** Because our microdissected tumor samples should have contained few dendritic cells and RT-PCR identified transcripts in cervical tissues and cell lines, we hypothesized that CD83 may have a role in cancer cells. Therefore, we investigated expression of CD83 protein using a specific monoclonal antibody in two immortalized normal cervical epithelial cell lines (CRL2614, ectocervix; CRL2615, endocervix), six cervical cancer cell lines (HeLa, CaSki, ME180, SiHa, HT3, and C4I), two ovarian cancer cell lines (CAVO3 and SKOV3), and one breast cancer cell line (MCF7). A weak CD83 signal was detected in all the cell lines tested, but signal intensities and locations differed. Double staining with CD83 monoclonal antibody (green) and nucleus-specific DAPI (blue) revealed traces of staining in the cell membrane and perinuclear staining in the cytoplasm. Colocalization staining was performed on cell lines HeLa, CaSki, ME180, CAVO3, SKOV3, and MCF7 using golgin-97 monoclonal antibody (red). It revealed that CD83 localized in the Golgi apparatus in HeLa and CaSki cells, in the cytoplasm but not in the Golgi apparatus in SKOV3 cells, in both the cytoplasm and adjacent membrane in ME180 cells, and in adjacent membrane in CAVO3 cells (Fig. 3). These results clearly confirm the presence of intracellular CD83 in all the cell lines tested.

**Polymorphisms and mutations in NOL7.** NOL7 has been shown to suppress tumor growth in a cervical cancer mouse xenograft model and is located 0.5 kb from the peak association and LOH region (25). In addition, to evaluate expression for NOL7 as noted above, we evaluated this gene for mutations/polymorphisms in 12 women with ICC. We found several new polymorphisms in exon 1, 3'-untranslated region (UTR), and introns and three somatic mutations in exon 1 in two of the ICC samples. The first mutation was G26A, which created a new start codon, ATG, upstream of the start ATG and produced a frameshift. The putative transcript encodes a protein containing 257 amino

### Table 1. Significant SNPs in discovery and validation sets for association study within 6p23

<table>
<thead>
<tr>
<th>SNP</th>
<th>All family trios</th>
<th></th>
<th></th>
<th>Family trios with HPV16 and HPV18 types</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>ICC (n = 121)</td>
<td>CIN3 (n = 122)</td>
<td></td>
<td>ICC (n = 98)</td>
</tr>
<tr>
<td></td>
<td>Discovery (n = 255)</td>
<td>Validation (n = 255)</td>
<td></td>
<td>Discovery (n = 184)</td>
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<td>0.1534</td>
<td>0.72441</td>
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</tbody>
</table>

![Figure 1.](image_url)
acids, whereas the mutated one would encode a protein of 59 amino acids. The second mutation was C141A, which causes a nonsynonymous change, Leu37Ile. The third was G254A, which caused a synonymous change, Ala74Ala (Table 2).

Discussion

Our data show that five SNPs within the 3′-end of CD83 are overtransmitted in women with ICC in a family-based association study, and thus, CD83 may represent a genetic risk factor for the disease, particularly in women with high-risk HPV16- and HPV18-related type infections. This interaction with HPV types parallels our previous study showing that the HLA DQB1 0303 allele is associated with an increased risk of cervical cancer in women with tumors containing HPV16, HPV18, HPV31, or HPV33 (6). No significant differences were found in the distribution of the CD83 alleles among women with HPV16- and HPV18-related cancer compared with those who had other HPV types, indicating that these SNPs are not associated with susceptibility to HPV16- or HPV18-related type infection. In this study, the association with invasive cancer but not with CIN3 further suggests that CD83 might have an effect later in carcinogenesis. Our recent publication of genomic signatures of cervical carcinogenesis showed that CIN3/squamous carcinoma transition coincided with a proinvasive gene signature and could be responding to the increased activity seen in the stroma at CIN2/3 (26). However, the limited number of CIN3 sample may not be statistically sufficient to rule out the association of CD83 with development of CIN3. Clearly, this needs to be evaluated in further studies.

Table 2. Previously unidentified mutations and polymorphisms in cervical tumors in genes NOL7 and CD83

<table>
<thead>
<tr>
<th>Gene</th>
<th>Location</th>
<th>Frequency in the samples</th>
<th>Nucleotide position*</th>
<th>Nucleotide change †</th>
<th>Amino acid change</th>
<th>Predicted effect</th>
<th>Chromosome 6 position ‡</th>
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<tbody>
<tr>
<td>NOL7</td>
<td>Exon 1</td>
<td>1/12</td>
<td>26</td>
<td>G&gt;A</td>
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<tr>
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<td>Exon 5</td>
<td>1/27</td>
<td>2224</td>
<td>A/G</td>
<td>3′-UTR</td>
<td></td>
<td>14244874</td>
</tr>
</tbody>
</table>

Abbreviation: n/a, not applicable.

* Nucleotide position for CD83 is based on NM_004233; for NOL7, it is based on NM_016167.
† Nucleotide change is compared with matched normal genomic DNA.
‡ Some tumor is T>C; some C>T.
\[ This mutation was found in cervical cancer cell line SW756.

Table 3. Expression of CD83 transcripts in cervical tissue and cell lines as determined by RT-PCR

<table>
<thead>
<tr>
<th>Cervical cell lines or cervical tissue</th>
<th>CD83-1</th>
<th>CD83-2</th>
<th>CD83-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal cervix (epithelium)</td>
<td>7/11</td>
<td>3/11</td>
<td>0/11*</td>
</tr>
<tr>
<td>Cervical cancers</td>
<td>7/8</td>
<td>5/8</td>
<td>4/8</td>
</tr>
<tr>
<td>Cervical cancer cell lines</td>
<td>12/12</td>
<td>6/12</td>
<td>4/12</td>
</tr>
<tr>
<td>CRL2614 (HPV-transformed ectocervix)</td>
<td>1/1</td>
<td>1/1</td>
<td>1/1</td>
</tr>
<tr>
<td>CRL2615 (HPV-transformed endocervix)</td>
<td>1/1</td>
<td>1/1</td>
<td>1/1</td>
</tr>
<tr>
<td>FC16 (HPV-transformed fetal cervical cell line)</td>
<td>1/1</td>
<td>0/1</td>
<td>0/1</td>
</tr>
<tr>
<td>9Ep12 (transformed CIN cell line)</td>
<td>1/1</td>
<td>0/1</td>
<td>0/1</td>
</tr>
</tbody>
</table>

* Lack of CD83-3 in normal cervical epithelium is significant (P = 0.0181) when compared with that in tumors.
Analyses of these data were carried out including all families in the study, although 341 of 377 families are Caucasian. The use of family-based controls, as in the transmission/disequilibrium test, allows us to pool over population groups because the methods are robust to the effects of population stratification (27). Nonetheless, we also repeated the analyses using only Caucasian families, and the results were qualitatively the same. In this study, we also showed that CD83 was transcribed and translated in cervical cancer cells. Somatic mutations in ICC, novel SNPs, and splice variants were also identified within CD83.

CD83 is an inducible glycoprotein belonging to the immunoglobulin superfamily. Its exact function is unknown, but it seems to have an important role in T-cell immunity mediated by dendritic cells and to be a marker for mature dendritic cells (28). However, recent literature suggests a broader functional role and to have an important role in T-cell immunity mediated by dendritic cells and to be a marker for mature dendritic cells (28).

Several mechanisms might potentially affect CD83 regulation or lead to inactivation. They include mutations, splice variants, allelic loss (LOH), and cancer-associated variants (Tables 2 and 3). dHPLC and sequencing confirmed LOH in the CD83 region because 21 of 23 (91%) tumors with informative SNPs had retained only one allele at specific SNP markers compared with matched normal genomic DNA. The sequence and structure of the 5’-UTR of the mRNA transcript is usually very important in regulating protein synthesis. Using electrophoresis mobility shift assays and deletion mutants, Berchtold et al. (36) narrowed down the highest promoter activity to the −261 bp region in CD83. This chromosomal fragment contains four SP1 binding sites and one NF-κB element. TNFα needs the latter to induce the CD83 promoter, and EBV needs it to induce CD83 expression on the surface of B cells (36, 37). One of 30 cervical tumors contained a mutation within the NF-κB element, and deletion polymorphisms were identified in the SP1 region (Table 2). The G170A SNP is nine nucleotides upstream of the translational start site (TSS), and loss of the G allele in cancerous versus normal cervix is highly significant (P < 0.025). In addition, 1 of 30 tumors had a single nucleotide deletion at −5 bp from the TSS (Table 2).

Prechtl et al. (38) identified a structural element—the posttranscriptional regulatory element (PRE)—in exons 4 and 5 of the CD83. PRE binds HuR (ELAVL1) and helps export CD83 mRNA from the nucleus. Binding of HuR to CD83 PRE does not affect the decay of CD83 transcripts but seems to alter the level of nucleocytoplasmic translocation via the CRM1 (XPO1) pathway. We identified three somatic mutations in exons 4 and 5. The 8-kb region containing the five significant SNPs lies within PRE and the 3’-end of CD83.

RNA splicing is essential for protein diversity. It can also have regulatory functions, and alternative splicing patterns can be cancer specific. The variant CD83-3 that lacks exons 3 and 4 was identified more often in cervical cancers and cell lines than in normal cervixes (P = 0.0181). Moreover, this alternative transcript is the only splice product secreted into the supernatant and elevated in sera from leukemic patients (39).

Our interest in the 6p23 region stems from extensive fine mapping of LOH in microdissected epithelial cervical cancer cells. Therefore, it may seem surprising that the current study identified an immune marker as a susceptibility gene for the disease. However, epithelial cells, once viewed principally as a mechanical
barrier, are now regarded as having roles that are more complex in cellular immunity. They contribute to the local inflammatory and immune responses by secreting or responding to various cytokines, growth factors, and chemokines. Toll-like receptors on cervical epithelial cells and dendritic cells provide the first-line response to pathogens by stimulating the production of defense proteins and mediating the influx of inflammatory cells into the infected site or cancer (40, 41). However, it is unclear whether CD83 could play a role in epithelial cells or dendritic cells or both. Further work may establish the mechanism for the increased expression of CD83 with ICC and determine which systems are involved.

NOL7 is a newly cloned gene, which located near our LOH region. A recent study showed that NOL7 showed a tumor-specific loss of a single allele. Transfection of NOL7 into cervical carcinoma cells inhibited their growth in mammalian xenografts by the decrease in the production of the angiogenic vascular endothelial cell growth factor, as an increase in the production of the naturally occurring inhibitor of angiogenesis thrombospondin-1 (25). Previous investigations and our newly identified somatic mutations make NOL7 a potential candidate tumor suppressor gene in late-stage development of cervical cancer.

HPV is an epitheliotropic virus that infects epithelial cells and subverts the immune response. However, infection with this virus must persist if a precursor lesion is to progress to ICC. Thus, escape from immune surveillance emerges as one important step in the progression of HPV-linked tumors (42). We have identified a candidate for genetic susceptibility that may act through immune responses. Clearly, functional assays and replication studies in independent cohorts are needed. Furthermore, identifying other genetic modifications to host cells that impair immune responses to HPV infection, permit infection to persist, and facilitate the progression to cancer will be important for the development of therapeutic interventions.

Acknowledgments


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References


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Zhengyan Zhang, Ingrid Borecki, Loan Nguyen, et al.


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