Gene expression profiling of peripheral blood mononuclear cells from women with cervical lesions reveals new markers of cancer

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Abstract. Cervical cancer (CC) is a multifactorial disease of which human papillomavirus (HPV) is the main etiological agent. Despite cervical Pap smear screening and anti-HPV vaccination, CC remains a major public health issue. Identification of specific gene expression signatures in the blood could allow better insight into the immune response of CC and could provide valuable information for the development of novel biomarkers. The present study performed a transcriptomic analysis of peripheral blood mononuclear cells (PBMCs) from Senegalese patients with CC (n=31), low-grade cervical intraepithelial neoplasia (CIN1; n=27) and from healthy control (CTR) subjects (n=29). Individuals in the CIN1 and CTR groups exhibited similar patterns in gene expression. A total of 182 genes were revealed to be differentially expressed in patients with CC compared with individuals in the CIN1 and CTR groups. The *IL1R2*, *IL18R1*, *MMP9* and *FKBP5* genes were the most upregulated, whereas the T-cell receptor α gene *TRA* was the most downregulated in the CC group compared with in the CIN1 and CTR groups. The pathway enrichment analysis of the differentially expressed genes revealed pathways directly and indirectly linked to inflammation. To the best of our knowledge, the present study is the first large transcriptomic study on CC performed using PBMCs from African women; the results revealed the involvement of genes and pathways related to inflammation, most notably the IL-1 pathway, and the involvement of downregulation of the T-cell receptor α, a key component of the immune response. Several of the stated genes have already been reported in other cancer studies as putative blood biomarkers, thus reinforcing the requirement for deeper investigation. These findings may aid in the development of innovative clinical biomarkers for CC prevention and should be further replicated in other populations.

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Introduction

Cervical cancer (CC) is the second most common type of cancer among women and a leading cause of cancer-associated death worldwide. Approximately 604,000 new cases of CC and 342,000 CC-associated deaths are reported each year, with ~80% of cases occurring in developing countries (1). In Senegal, it is the most common type of cancer after breast cancer, with 1,937 new cases, 1,312 deaths and a 5-year prevalence of 3,543 reported in 2020 (1).

Infection with human papillomavirus (HPV) is the main cause of CC and ~80% of sexually active women will be infected in their lifetime (2). However, >90% of infections are asymptomatic and will be cleared by the immune system within 6 months to 2 years (2). A persistent infection may first

lead to low or high-grade cervical intraepithelial neoplasia (CIN1 to CIN3), and then may evolve toward CC (2).

There are >100 types of HPV with various oncogenic potential (3). HPV-16 and -18 are the main types involved in 78% of CC cases (3). Other HPV types, such as HPV-31, -33, -35, -39, -45, -51, -52, -56, -58 and -59, are defined as high-risk HPV types according to the World Health Organization (4). The HPV genome encodes the proteins E6 and E7, which have an oncogenic action by stimulating cellular proliferation through the inactivation of regulatory proteins, including p53 or pRb (5). Besides HPV infection, several co-factors have been shown to be involved in malignant transformation, including sexually transmitted diseases, the age onset of sexual activity, menstruation and childbirth, smoking, immune deficiency and viral infections (6). In addition, host genetic variations are important in the development of CC (7).

CC prevention has successfully reduced mortality in developed countries through the development of screening tools for HPV and the HPV vaccine (8). The cytology test ('Pap smear') detects HPV-associated dysplastic changes in exfoliated cervical cells; however, it has a rather low sensitivity for detecting precancerous lesions and the screening has to be repeated at frequent intervals. The Pap smear test is widely used and remains highly effective for preventing death from CC (9). The HPV DNA test has a higher sensitivity and thus requires less follow-up tests than the Pap smear (10). In addition to screening tools, the HPV vaccine has exhibited a good efficacy in preventing infection and disease caused by specific HPV types (11). The success of prevention and vaccination is linked to the cost of effective public health policies, and there are major differences worldwide in terms of access to screening and vaccines (12). To tackle CC effectively, in addition to vaccination, screening has a major role. New biomarkers for CC, in addition to classic screening, are still required since they may help to improve the accuracy of screening and diagnosis, and thus improve the specific detection of cervical lesions, as well as early-stage CC, leading to a better efficacy of cancer treatment (13).

The emergence of 'omics' technologies, such as genomics, transcriptomics and proteomics, opens a new way of investigation for a better understanding of disease etiology and for the discovery of biomarkers for patients with cancer (14). Blood transcriptomics offers the possibility to evaluate the immune response in patients with cancer and may help to develop blood-based biomarkers, detection of which may be affordable in a low-resource and high disease-burden environment. Blood-based gene expression biomarkers have already been investigated in human cancer with promising results, but not in CC (15,16). In the present study, the mRNA expression in peripheral blood mononuclear cells (PBMCs) was obtained and compared among three categories of subjects: Patients with CC, patients with CIN1 and healthy control (CTR) subjects. Using the transcriptome profiles, the present study investigated the molecular mechanisms in PBMCs from patients with CIN1 and CC compared with CTR subjects, through the analysis of biological pathways.

Materials and methods

Study population. Peripheral blood samples were collected from 31 patients with CC, 27 patients with CIN1 and 29 healthy CTR subjects, and were placed in PAXgene Blood RNA tubes (PreAnalytiX GmbH). The patients were recruited from Senegal, at the Center of Cancerology, Aristide le Dantec Hospital (Joliot-Curie Institute, Dakar, Senegal) and at the Gaspard Camara Health Center (Dakar, Senegal) between January 2016 and December 2017. The CTR subjects included in the study were also assessed at the same hospital as the patients (Aristide le Dantec Hospital). Since only cancer cases are seen and treated at Aristide le Dantec Hospital, the CTR subjects were initially identified at the North Health Center of Yeumbeul (Dakar, Senegal), which provides regular consultations for women, and the women were addressed to the Aristide le Dantec Hospital to be included in the study if they agreed to participate. The clinical diagnosis of CC and CIN1 was confirmed by a histological examination of the biopsies and according to the inclusion criteria, the patients with CC and CIN1 did not exhibit other malignancies or serious medical conditions, and patients with CC did not receive chemotherapy, radiotherapy or hormonal treatment prior to surgery. The CTR subjects were composed of healthy women free from cancer and CIN (colposcopy and Pap smear were performed to control for CIN) and without a family history of cancer.

All subjects signed an informed consent form and the study was approved by the Ethical and Scientific Committee of Cheikh Anta DIOP University of Dakar (approval no. 0197/2016/CER/UCAD). Data on all the patients and CTR subjects collected included information on socio-demographic and epidemiological characteristics, such as age, marital status, type of marriage (polygamy vs. monogamy), cigarette smoking, alcohol consumption, reproductive history (menarche, parity and gravidity), sexual history (lifetime, number of sexual partners, age at first intercourse), family history of cancer, and medication and supplement use. The clinical and histopathological characteristics of the patients included in the present study are presented in Tables I and II.

Phenotype assessment. All women had a colposcopy and a Pap smear, to provide a new diagnosis for the presence or absence of dysplasia. Colposcopy and histology were performed for patients with CIN to assess the severity of dysplasia, and then patients with CIN2 (middle-grade) or CIN3 were removed to avoid CIN patients with cancer. Finally, women with CC had a gynecological examination, biopsy, pelvic and lumbo-aortic MRI, pulmonary radiography, thoraco-abdomino-pelvic scan, cystoscopy and rectoscopy. The pelvic and the lumbo-aortic MRI allows for assessment of cervical size, to study extension to the rectovaginal septum, to the uterine isthmus and to the lymph nodes, and evaluates renal integrity. The thoraco-abdomino-pelvic scan was performed to assess liver, lung and bone metastases, and peritoneal carcinomatosis. The cystoscopy and rectoscopy were performed in cases of suspected bladder and kidney damage.

Whole blood collection and RNA isolation. Whole blood samples (2.5 ml) were collected into PAXgene Blood RNA tubes and stored at room temperature for 3 h, to achieve complete lysis of the blood cells, and immediate and persistent RNA stabilization. The PAXgene Blood RNA Kit (PreAnalytiX GmbH) was used to isolate PBMCs from the

Table I. Clinical features of CC, CIN1 and CTR groups.

Characteristic	CC (n=31)	CIN1 (n=27)	CTR (n=29)
Age range, years	28-75	25-59	20-61
Median age, years	53.58	42.66	29.65
Marital status			
Polygamy, % (n)	67.74% (21)	37.04% (10)	10.34% (3)
Monogamy, % (n)	32.26% (10)	62.96% (17)	27.59% (8)
Unmarried, % (n)	0% (0)	0% (0)	62.07% (18)
Tobacco smoking			
Yes, % (n)	0% (0)	3.70% (1)	3.45% (1)
No, % (n)	100% (31)	96.30% (26)	96.55% (28)
Alcohol consumption			
Yes, % (n)	0% (0)	0% (0)	0% (0)
No, % (n)	100% (31)	100% (27)	100% (29)
Oral contraceptive use			
Yes, % (n)	22.58% (7)	85.19% (23)	24.14% (7)
No, % (n)	77.42% (24)	14.81% (4)	75.86% (22)
Gravidity			
<5, % (n)	19.35% (6)	59.26% (16)	86.21% (25)
≥5, % (n)	80.65% (25)	40.74% (11)	13.79% (4)
Parity			
<5, % (n)	22.58% (7)	77.78% (21)	89.66% (26)
≥5, % (n)	77.42% (24)	22.22% (6)	10.34% (3)

CC, cervical cancer; CIN1, cervical intraepithelial neoplasia; CTR, control.

blood and to then extract RNA, according to the manufacturer's protocol. RNA quality was determined by detecting 28S/18S rRNA peaks with an Agilent Bioanalyzer 2100 (Agilent Technologies, Inc.). Total yield of RNA (ng) was determined using a NanoDrop ND-1000 spectrophotometer (NanoDrop; Thermo Fisher Scientific, Inc.). All samples presented an RNA integrity number of >7.0 and a 28S:18S rRNA ratio of >1.0.

Amplification and hybridization. Total RNA was reverse transcribed, amplified and purified using TargetAmp™-Nano-g Biotin-aRNA Labeling Kit for Illumina® Expression BeadChip® (cat. no. TAN07924-142; Epicentre; Illumina, Inc.). Briefly, 100 ng RNA was reverse transcribed to synthesize the first strand of cDNA using the reverse transcriptase SuperScript III (Invitrogen; Thermo Fisher Scientific, Inc.) with the kit TargetAmp™-Nano-g Biotin-aRNA Labeling Kit for Illumina® Expression BeadChip® (cat. no. TAN07924-142; Epicentre; Illumina, Inc.). The mixture was heated to 65˚C for 5 min and incubated on ice for ≥1 min. This step was followed by second-strand synthesis using the thermocycler 9600 (QuantGene, Inc.) (65˚C for 10 min, then 80˚C for 3 min, followed by cooling on ice and maintenance at -80˚C). Subsequently, double-stranded cDNA was transcribed and amplified *in vitro* to synthesize biotin-labeled complementary mRNA (cRNA) by incorporating biotin-CTP and biotin-UTP. The cRNA yield was measured at 260 nm using the NanoDrop ND-1000 spectrophotometer. Finally, 750 ng of cRNA per sample was hybridized with beads using Illumina Bead Chip Human 6v2 Arrays or a Human HT-12 Expression Beadchip (Illumina, Inc.) profiling 48,701 transcripts per sample. The chips were stained with streptavidin-Cye3 conjugate.

Data acquisition and preprocessing. Beadchips were scanned on the Illumina BeadArray 500GX reader using the Illumina BeadScan image data acquisition software (version 2.3.0.13; Illumina, Inc.). Illumina GenomeStudio software (version 2.0.4; Illumina, Inc.) was used for preliminary data analysis to validate the experiments. Several quality metrics were evaluated for each run: Variations in signal intensity, hybridization signal, background signal and the background/noise ratio for all the samples analyzed. GenomeStudio was then used to compute the expression values as log2 ratios of the fluorescence intensities (experimental/common reference sample) and to normalize the expression values using the 'normalize quantiles' option. The normalized data were inspected using principal component analysis (PCA) [prcomp from the Stats R Package (17)] in order to detect outlier samples (Fig. S1).

Statistical methods. Differentially expressed genes (DEG) across CC vs. CIN1, CC vs. CTR and CIN1 vs. CTR comparisons were identified from log2-transformed normalized expression values using Linear Models for Microarray Data (LIMMA, package version 3.48.0) and the unpaired moderated t-test (18). The moderated t-test differs from the Student's t-test through the calculation of an adjusted variance based on the

Table II. Clinical characteristics of patients with cervical cancer (n=31).

Characteristics	% (n)
Tumor stage [FIGO (52)]	
IIA	9.68% (3)
IIB	29.03% (9)
IIIA	6.45% (2)
IVA	38.71% (12)
IVB	6.45% (2)
Unknown	9.68% (3)
Tumor differentiation	
Well	41.94% (13)
Moderate	48.38% (15)
Poor	0% (0)
Unknown	9.68% (3)
Lymph node metastases	
Positive	6.45% (2)
Negative	93.55% (29)
Tumor size, cm	
<4	9.68% (3)
≥4	90.32% (28)
Infiltration depth, mm	
<15	9.68% (3)
≥15	90.32% (28)
Vascular invasion	
Yes	90.38% (28)
No	0% (0)
Unknown	9.68% (3)

variance of all genes analyzed. In order to take into account the multiple testing across the three comparisons, the 'global' method from LIMMA was used that applies a false discovery rate (FDR) correction (Benjamini-Hochberg procedure) to all the tests together regardless of which probe or comparison they relate (18,19). As a secondary analysis, the gene expression was compared between cancer at low stages (I and II, n=12) with that of cancer at high stages (III and IV, n=16) using LIMMA and the unpaired moderated t-test (18). The FDR (Benjamini-Hochberg procedure) was applied independently for this comparison (19). For all comparisons, a gene was considered as a DEG with P_{adj}<0.05 (P_{ajd} obtained with FDR correction) and log2 fold change (log2FC) >|1|.

Hierarchical clustering of the log2-transformed normalized data was performed with the hclust from the Stats R Package R package (17) on the 182 significant genes (P_{adj}<0.05, log2FC >|1|). In order to functionally determine these clusters, a Gene Set Enrichment Analysis (GSEA) was performed to determine the enriched Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways and Gene Ontology (GO) terms for each cluster, using the Search Tool for the Retrieval of Interacting Genes database 5 (https://string-db.org/version11.0) (20). The top ranked KEGG pathways and GO terms were selected to characterize the clusters.

Results

Patients. To explore the gene expression profile of immune cells in patients with CC and CIN1, a transcriptomic analysis was performed by comparing whole blood RNA from the PBMCs of 31 patients with CC, 27 patients with CIN1 and 29 CTR subjects. All patients with CC had been diagnosed with squamous cell carcinoma (100%), and were in stage IIA (9.68%), IIB (29.03%), IIIA (6.45%), IVA (38.71%) and IVB (6.45%) at the time of diagnosis (Table II). Expression data for 7,094 genes were available for differential expression analysis after quality control.

PCA and a hierarchical clustering of samples were performed to ensure the quality of the data. For the PCA, one cluster was detected for CC, and one cluster was detected for CIN1 and CTR, separated according to the first principal component of the PCA (Fig. S1). The same groups were observed in the hierarchical clustering; however, the CTR sample 027 was more related to patients with CC and the CC sample 020 was more related to CTR subjects (Fig. S2). In the PCA, these samples were at the limit of the two clusters, thus they were not removed from the analysis (Fig. S1).

As shown in Table I, there were differences between CTR, CIN1 and CC samples regarding age, marital status, oral contraceptive use, gravidity and parity showing the importance of adding these parameters as covariates in the differential gene expression analysis.

Gene expression profiles. A total of 182 DEGs were obtained for the CC vs. CIN1 and CC vs. CTR comparisons (P_{adj}<0.05, log2FC>|1|; Fig. 1; Table SI). By contrast, no significant DEGs (P_{adj}<0.05, log2FC>|1|) were observed between CIN1 and CTR (Table SI), nor between cancer at low stages (I and II) and cancer at high stages (III and IV) (data not shown).

Similar DEG profiles were observed for the CTR vs. CC and CIN1 vs. CC comparisons (Fig. 1). Notably, 136 genes were upregulated in patients with CC, with 98 upregulated compared with patients with CIN1 and 117 upregulated compared with the CTR subjects. In addition, there were 46 genes downregulated in patients with CC, with 42 downregulated compared with patients with CIN1 and 11 downregulated compared with the CTR subjects. A number of the genes that were not found in both the CTR vs. CC and CIN1 vs. CC comparisons were still significant with similar trends, but with a log2FC<|1|. The genes exhibiting the highest log2FC and the strongest P-values for the CC vs. CTR and CC vs. CIN1 comparisons were *IL1R2*, *MMP9*, *IL18R1* and *FKBP5* (Fig. 2; Table SI). The genes exhibiting the lowest log2FC and the strongest P-values were *TRA*, *CD27* and *STMN3* for the CC vs. CTR comparison, and were *RPL38*, *FLT3LG* and *TRA* for the CC vs. CIN1 comparison (Fig. S3). The *TRA* gene exhibited the lowest log2FC and the strongest P-values for the two comparisons. The strongest log2FC values were found for genes upregulated in CC.

Clustering and GSEA. The 182 significant DEGs across the three comparisons were analyzed by hierarchical clustering, in order to detect expression pattern, and co-regulated and functionally related genes. Subsequently, a GSEA was performed on the KEGG pathways and GO terms for each cluster.

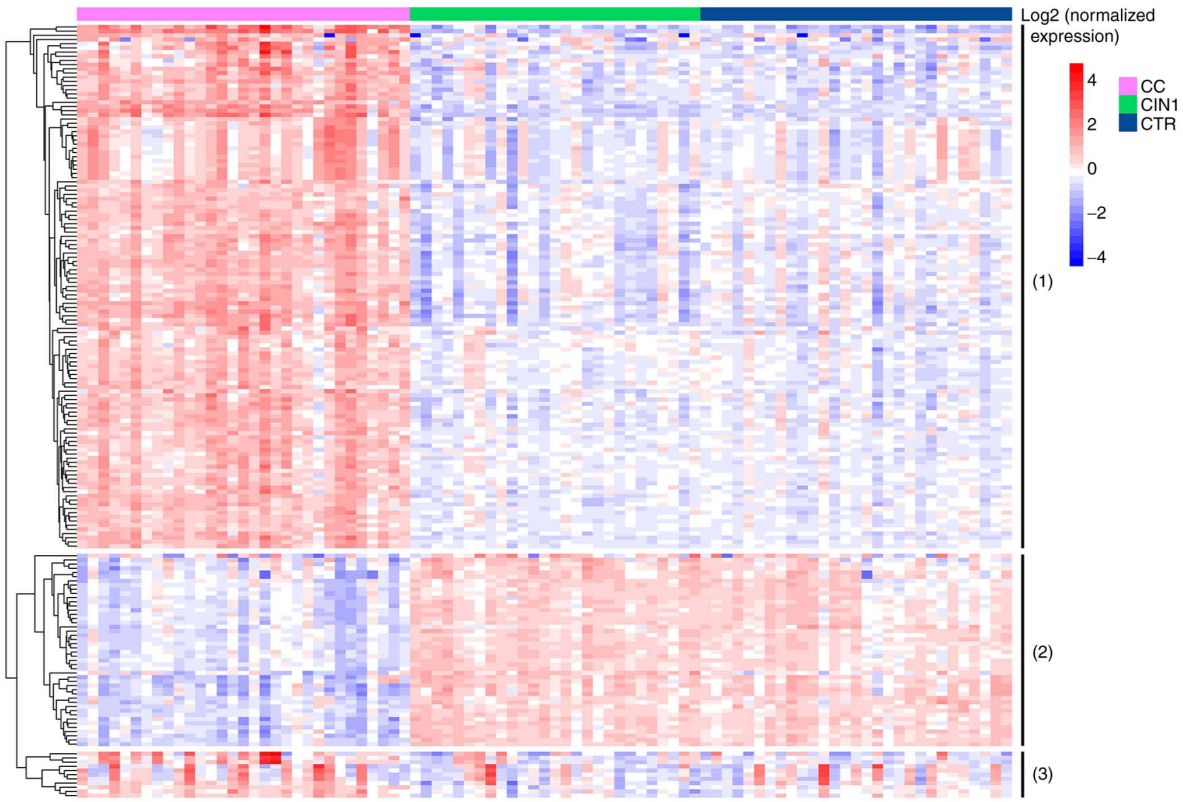

Figure 1. Heatmap of differentially expressed genes between CC (n=31), CIN1 (n=27) and CTR (n=29) samples obtained by supervised hierarchical clustering. Differentially expressed genes were obtained by unpaired t-test. Genes are organized by hierarchical clustering based on overall similarity in expression patterns. This analysis was performed on log2-transformed normalized expression data. The rows are the genes; red represents relative expression greater than the median expression level across all samples, and blue represents an expression level lower than the median. White indicates intermediate expression. The clusters are numbered on the right of the heatmap. CC, cervical cancer; CIN1, cervical intraepithelial neoplasia; CTR, control.

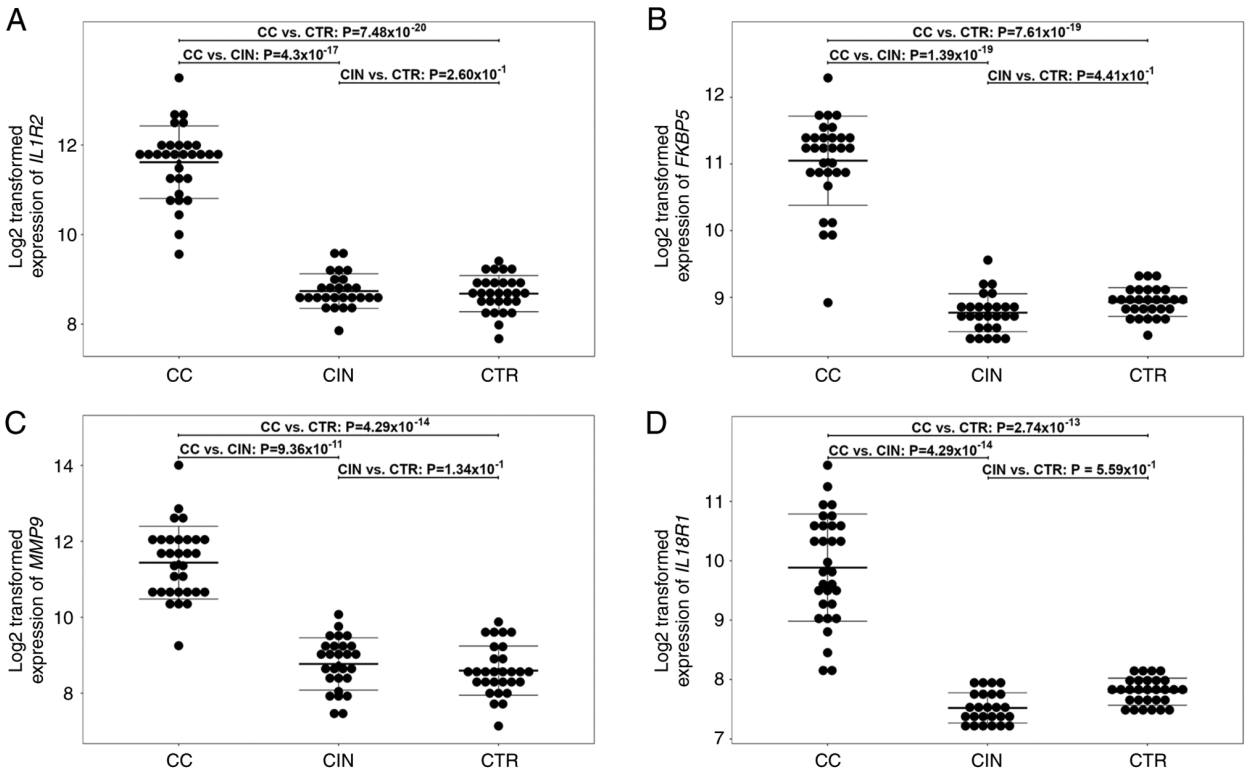

Figure 2. Dot plots of log2-transformed normalized expression of the genes exhibiting the highest log2FC and the strongest P-values for the CC vs. CTR and CC vs. CIN1 comparisons. Differentially expressed genes were identified from log2-transformed normalized expression values using the Linear Models for Microarray Data and a moderated t-test. (A) Expression levels of the *IL1R2* gene. (B) Expression levels of the *FKBP5* gene. (C) Expression levels of the *MMP9* gene. (D) Expression levels of the *IL18R1* gene. CC, cervical cancer; CIN1, cervical intraepithelial neoplasia; CTR, control; FC, fold change.

Three main clusters (1-2-3) were distinguished (Fig. 1). Cluster 1 was characterized by an enrichment of genes upregulated in patients with CC compared with in individuals in the CIN1 and CTR groups. The analysis of cluster 1 revealed an enrichment of genes included in the KEGG pathways 'IL-17 signaling pathway' and 'inflammatory bowel disease' (Table SII). Cluster 2 was composed of genes with lower expression in patients with CC compared with in individuals in the CTR and CIN1 groups. The analysis of cluster 2 revealed an enrichment of genes belonging to the 'ribosome' and 'cytokine-cytokine receptor interaction' KEGG pathways (Table SII). There were few genes in cluster 3 and they were upregulated in patients with CC compared with in individuals in the CIN1 and CTR groups; however, the FCs were larger in the comparison with CIN1. There was no KEGG pathway enriched for this cluster. The results of the enrichment analysis in GO terms were similar to those obtained for KEGG pathway analysis for clusters 1 and 2 (Table SIII). Notably, for cluster 1, GO terms related to immunity were obtained: 'macrophage activation' and 'neutrophil aggregation'. For cluster 2, GO terms related to DNA translation and immunity were enriched: 'SRP-dependent cotranslational protein targeting to membrane', 'nuclear-transcribed mRNA catabolic process, nonsense-mediated decay', 'viral transcription', 'translational initiation', 'establishment of protein localization to membrane', 'cytoplasmic translation', 'ribosomal small subunit assembly', 'ribosomal small subunit biogenesis', 'positive regulation of lymphocyte differentiation' and 'positive regulation of lymphocyte activation' (Table SIII). The results for KEGG and GO enrichment were similar and we obtained common genes in the pathways from KEGG and the terms from GO (Tables SII and SIII). Genes in cluster 3 were enriched in GO terms related to the immune response, specifically 'response to virus' and 'cytokine-mediated signaling pathway' (Table SIII).

mRNA expression in the blood in other cancer studies. Since blood-based biomarkers have become a major asset to detect cancer, there have been several studies in this field regarding various types of cancer. Zuo *et al* (21) developed a web-accessible and comprehensive open resource database to provide the mRNA expression landscape in blood. The present study compared the set of top ranked genes in the blood of patients with CC (genes exhibiting the highest log2FC and the strongest P-values: *IL1R2*, *MMP9*, *IL18R1* and *FKBP5*) and the genes belonging to the enriched KEGG pathways ('IL-17 signaling pathway', 'inflammatory bowel disease', 'ribosome' and 'cytokine-cytokine receptor interaction'; Table SII) to those dysregulated in the PBMCs of patients with colorectal cancer (988 genes with P<0.05) or lung cancer (779 genes with P<0.05) available in this database. The gene *TLR5* was upregulated in patients with CC and was also upregulated in patients with colorectal cancer. By contrast, the gene *IL8RAP* was upregulated in patients with CC and downregulated in the blood of patients with lung and colorectal cancer. Additionally, Ma *et al* (22) recently published an RNAseq study in the blood of patients with CC (n=11) and CIN1 (n=21) compared with CTR subjects (n=19) from China, and identified nine significant genes that were confirmed by reverse transcription-quantitative PCR in 83 CC, 32 CIN1 and 46 CTR samples (*AGAP1*, *CDC42EP2*, *GPR84*, *GZMB*, *KIF19*, *NUAK1*, *CIR1*, *DNAJA1* and *NDUFA1*) (22). These nine genes were searched among the entire transcriptomic results of the present study but five were absent from the analysis (*AGAP1*, *CDC42EP2*, *GRP84*, *KIF19* and *NUAK1*) and the other four were not significant (*GZMB*, *CIR1*, *DNAJA1* and *NDUFA1*; P_{adj}>0.05, log2FC<|1|). We could not test the results obtained for the 182 DEGs in this previous study, since the data were not available.

Discussion

Transcriptomic studies are a useful tool for assessing gene expression levels in cancer, which can help improve the understanding of disease etiology and discover possible biomarkers (23). The transcriptomic analysis of PBMCs offers a novel resource for a better understanding of the interactions between the immune response and cancer cells, and eventually for the identification of non-invasive tumor biomarkers (15,16).

The present study explored the transcriptional profiles of whole blood samples obtained from 31 patients with CC, 27 patients with CIN1 and 29 CTR individuals. Microarray technology has been used to discriminate differences in gene expression profiles in PBMCs. The present study identified several genes, the expression levels of which differed between the CIN1, CC and CTR groups at P_{adj}<0.05, log2FC>|1|.

No DEGs were observed between the CIN1 and CTR groups. A power analysis by sample size was performed, which indicated that the number of individuals in the present study was largely sufficient to detect genes differentially expressed between the CIN1 and CTR groups (data not shown). It has been reported that the levels of pro-inflammatory cytokines in cervical tissues, such as IL-1, are higher in CIN compared with CTR, and are even higher in CIN3 compared with CIN1 (24). Since the present study assessed PBMCs, it may be hypothesized that the inflammation in CIN1 remains local and smaller compared with high grade CIN, thus no difference was observed at the level of PBMCs when comparing CIN1 with CTR and explaining the absence of significant DEGs. The lack of DEGs between CIN1 and CTR suggested low or no dysregulation of gene expression in patients with CIN1. The lack of significant differences between the cancer stages (low vs. high) may be explained by the small sample size or an earlier impact of the identified genes on carcinogenesis, thus leading to less and finally no strong differences in gene expression in PBMCs between the cancer stages. The absence of DEGs between the CIN1 and CTR groups, and between cancer stages should be confirmed in an independent cohort.

The present study identified 182 genes for which expression differed between CC, CIN and CTR groups. The genes with the highest fold changes and the strongest P-values in CC compared with CTR or CIN1 were *IL1R2*, *MMP9*, *IL18R1* and *FKBP5*. Notably, the proteins encoded by *IL1R2* and *IL18R1* are cytokine receptors that belong to the IL-1 receptor family and to a gene cluster on chromosome 2q12. IL-1R-2 binds IL-1α, IL-1β and IL-1R-1/IL-1RN, and acts as a decoy receptor that inhibits the activity of IL-1, whereas IL-18R-1 binds IL-18 and IL-18RAcP activating similar pathway as that of IL-1 (25,26).

Genetic variations of *IL1R2* have been shown to be associated with the risk of CC and gall bladder cancer (27,28). Moreover, several studies in various types of cancer have reported a difference in the expression of *IL1R2* in tumor tissue or in the tumor microenvironment, and it has been reported as a potential prognostic or therapeutic target in several analyses (29,30). *IL18* has been identified as a putative contributor to viral pathogenesis or carcinogenesis in CC (31). Additionally, the IL-18 pathway may have a role in immunotherapeutic intervention in cancer (32). The present results on the IL-1 superfamily are in agreement with previous results, and emphasize the importance of investigating these genes and the IL-1 pathway as potential biomarkers or for immunotherapies in CC. The *MMP9* gene is located on chromosome 20q13 and is involved in proteolytic degradation of the extracellular matrix (ECM), alterations in cell-cell and cell-ECM interactions, cleavage of cell surface proteins and cleavage of proteins in the extracellular environment (33). Since several important processes of carcinogenesis are related to the extracellular environment, MMP-9 has been widely associated with cancer pathologies. In the case of CC, *MMP9* expression has been reported to be elevated in tumor tissue and in the plasma of patients with CC in two other studies (34,35). These studies identified *MMP9* as a useful biomarker in the diagnosis of CC in combination with other biomarkers. The protein encoded by *FKBP5* located on chromosome 6p21 is a member of the immunophilin protein family. It has been demonstrated that *FKBP5* may serve a significant role in modulating rapamycin treatment resistance and could improve the sensitivity of rapamycin-resistant cells to rapamycin treatment for cancer (36). The gene with the lowest fold changes and highest P-values for the CC vs. CTR or vs. CIN1 comparisons was *TRA*. *TRA* encodes the T-cell receptor α which is a part of the αβ T-cell receptor (TCR) complex. The TCR complex is expressed at the surface of T cells and is responsible for recognizing fragments of antigen bound to major histocompatibility complex molecules for the activation of the T-cell response (37). This association is thus very relevant to cancer since tumor escape from immune destruction has been widely described (38). It would be very interesting to understand the molecular etiology of this modulation of TCR expression in CC. Notably, TCR cell therapies are currently being tested in several types of advanced cancer indicating that this technology is likely safe and prospectively efficacious (39).

In the present study, clustering and GSEA allowed the identification of pathways and genes that may be altered in patients with CC. For genes with a higher expression in CC compared with in CIN1 and CTR groups, genes were revealed to be enriched in 'IL-17 signaling pathway' and 'inflammatory bowel disease'. IL-17 is the founding member of a novel family of inflammatory cytokines. Notably, it has been shown that IL-17 can promote the migration and invasion of CC cells by upregulating *MMP2* and *MMP9* expression, and downregulating *TIMP1* and *TIMP2* expression via the p38/NF-κB signaling pathway (40). This result could reflect the inflammatory state of patients with CC and the role of inflammatory cytokines in response to cancer. The 'inflammatory bowel disease' pathway is characterized by chronic inflammation of the gastrointestinal tract due to environmental and genetic factors, infectious microbes and the dysregulated immune system. This result may be related once again to the chronic inflammation of patients with cancer. For genes with a lower expression in CC compared with in CIN1 and CTR groups, pathways related to 'ribosome' and 'cytokine-cytokine receptor interaction' were enriched. The ribosome is an intracellular organelle, consisting of RNA and protein, which is a factory for the production of proteins by translation of mRNA (41). The link between the ribosome and the innate immune response has been established and it has been reported that translational inhibition could trigger inflammation through IL-1β signaling (42). Thus, the downregulation of genes related to the ribosome in the patients with CC in the present study could reflect the chronic inflammatory state of these patients. The 'cytokine-cytokine receptor interaction' pathway contains cytokines and their receptors grouped by structure into different families. Dysregulation in circulating cytokine levels has been reported to be associated with the presence of numerous types of cancer (43). For example, IL-6 levels are associated with renal cell carcinoma metastasis. The genes *CD27*, *CCR7*, *CXCR5* and *LTB*, belonging to the 'cytokine-cytokine receptor interaction' pathway, deserve deeper investigation and could provide information on circulating cytokines of interest in CC.

The analysis of gene expression in PBMCs in other types of cancer has identified *TLR5* and *ILRAP18* genes (21). Notably, these two genes belong to the 'inflammatory bowel disease' pathway, confirming the importance of immune system deregulation. More specifically, comparison of the present findings with those of an RNAseq study in PBMCs from Chinese patients with CC (22) revealed that the significant results of the previous study were not replicated in the present analysis. This discrepancy could be explained by several differences in the methods, including the number of patients in the two cohorts, the techniques used to measure gene expression (RNAseq/cDNA microarray) and the geographical origins of the patients (44).

In the present study, the results were mostly related to inflammation. There are several lines of evidence indicating that inflammation likely has a role in HPV-associated carcinogenesis (45), and this is confirmed by the present results. A recent study also demonstrated that the levels of circulating inflammatory markers were significantly increased in patients with HPV-positive CC compared with those in healthy controls (46). These results and those of the present study underline the high levels of circulating inflammatory cytokines in patients with CC and thus the systemic inflammation (47). Several investigations have been performed on the possible use of non-steroidal anti-inflammatory agents (NSAIDs) or steroids as preventive or as therapeutic treatment for CC. The effectiveness of NSAIDs to induce regression and prevent the progression of CIN toward cancer provided no convincing data to support the benefit for NSAIDs in the treatment of CIN (48). The use of NSAIDs or steroids as therapeutic drugs has been tested in several types of cancer, and studies have highlighted the antitumor effects of the two types of anti-inflammatory molecules in CC (49-51). The present results emphasized the interest of investigating the role of anti-inflammatory drugs as possible treatment strategies in CC.

To the best of our knowledge, the present study is the first transcriptomic study assessing PBMCs in African patients

with CC; notably, this population may be at a higher risk of CC as the HPV vaccine is not widely available. The present study detected several dysregulated genes and pathways in patients with CC compared with in CTR individuals and patients with CIN1. These two latter groups exhibited a highly similar gene expression profile, whereas the demographics (e.g. parity, gravidity) of the patients with CIN1 were much closer to the patients with CC, which strengthens these results. Globally, the results pointed directly or indirectly to inflammation, and notably to the IL-1 pathway. These findings could reflect the dysregulation of proinflammatory cytokines in the PBMCs of patients with CC and thus to an underlying inflammatory/immunological disorder. Notably, the association with the ribosome system identifies novel fields of investigation for CC. The use of PBMCs in the present study offers an approach to understand the immune response in CC. It will be necessary to perform replication and further investigations to better identify the specific role of various blood cell types (e.g. granulocytes, monocytes, lymphocytes) and to better understand the mechanisms at stake. As for any genomic study, the results obtained in the present study require replication in independent cohorts in order to be fully validated. All of the genes and pathways identified as relevant in the present study deserve additional investigations to confirm their role and explore their potential as therapeutics or as biomarkers.

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Availability of data and materials

The datasets generated and/or analyzed during the current study are available in the 'cervical_cancer' repository (accession number: TF-1210-TL), http://griv.org/cervical_cancer/.

Authors' contributions

MN, GD, MT, MS and AD conducted the research and investigation process, specifically designing the study and performing the data collection. CD, MN and JFD performed the genomics experiments. CC, JLS, RMS and SLC performed the statistical analysis of the data. MN, SLC, JN, AT and JFZ participated in the analysis and the interpretation of the results. MN, SCL and JFZ wrote the draft of the paper. JN, RMS and AT provided a critical review of the draft. All authors have contributed to, and have read and approved the final manuscript.

Ethics approval and consent to participate

All patients signed the informed consent form and the study was approved by the Ethical and Scientific committee of Cheikh Anta Diop of Dakar University (approval no. 0197/2016/CER/UCAD).

Patient consent for publication

Not applicable.

Competing interests

The authors confirmed they had no competing interests.

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