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The Deficiency of Nuclear Proteins GATA6 and Lamin A/C as Prognostic Factor for Cervical Neoplasia

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Abstract **BACKGROUND**: Cervical cancer is a deadly disease for women worldwide and could be prevented if early molecular screening was performed ahead of cytology. In most neoplastic lesions the expression of nuclear proteins GATA6 and/or lamin A/C is deficient. OBJECTIVE: To analyze GATA6 and lamin A/C in archived cervical epithelial tissues and in cells derived from cervical uterine smears (CUS). METHODS: Immunofluorescence was used to analyze GATA6 and lamin A/C in epithelial cell lines. Immunohistochemistry was used to analyze GATA6 and lamin A/C expression in normal and neoplastic archived cervical tissues. Immunoblotting was used to analyze GATA6 and lamin A/C in cells of CUS obtained freshly from 40 selected women (age 20-45 years) who were attending routine gynecological checkup in Hospital Mènontin (Cotonou, BENIN). Colposcopy was used to visualize the vaginal tract and the uterine-cervix junction. This study is approved by our Institutional Research Ethic Committee and by the Ministry of Health in BENIN. RESULTS: Our data shows that the expression of GATA6 and lamin A/C is altered prior to cervical neoplastic lesions. The nuclear biomarkers GATA6 and lamin A/C are absent in 13% of CUS samples. Colposcopic examination performed after two years on 15 women lacking GATA6 and/or lamin A/C shows that 20% of them have developed cervical neoplastic lesions. CONCLUSION: These preliminary results suggest that the deficiency of GATA6 and lamin A/C in CUS could be used as biomarkers to contribute to the identification of women at risk of developing cervical lesions. However further studies are required for cancer prevention.

Keywords: *nuclear proteins, GATA6, lamin A/C; cervical neoplasia, cancer prevention*

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1. Introduction

Cervical cancer is one of the most deadly affliction among women worldwide [1,2,3,4] and could be prevented if reliable molecular biomarkers are discovered beside HPV infection to identify molecular perturbations prior to the cell morphological transformation used by pathologists to screen cancer cells [3,5,6]. Research teams over the last decade demonstrated that the nuclear and epithelial differentiation proteins GATA6 and lamin A/C have role in the maintenance of nuclear architecture and the differentiated state of epithelial cells. Their deficiency initiates cell dedifferentiation, cell cycle failure, polyploidy, aneuploidy and chromosomal instability, all of which are hallmarks of cancer cells as reported previously [6,7,8,9,10].

GATA6 transcription factor initiates the embryonic stem cell differentiation and maintains the differentiated shape of adult epithelial tissues [5,6,7,11,12]. The loss of GATA6 in ovarian cancer is associated to undifferentiated cells, disorganized epithelium and reduced expression of nuclear envelope proteins including lamin A/C [5,6,7,8,9]. Subsequently, atypical nuclear morphology and chromosomal aberration are observed [7,8,13].

LMNA gene encodes nuclear envelope proteins Lamin A/C (A-type lamins) which are intermediate filaments expressed in many differentiated cells and constitute the scaffold of the nuclear envelope which is involved in the control of cell cycle progression, and the stable segregation of chromosomes [8,9,14,15]. Cells bearing mutation in LMNA gene display abnormal nuclear morphology, defective cell cycle kinetics, polyploidy, loss of heterochromatin organization and chromosomal

aberration [16,17]. The lack of lamin A/C observed in ovarian and breast cancer cells is associated with altered nuclear structure, chromosomal instability and mitotic failure [8,9]. Viral infections (HPV) are mostly known to cause cervical cancers but other factors are also involved [18,19]. Undeniably, the majority of cervical cancers originated from the squamous epithelium and transformed cells have all the molecular perturbations emphasized overhead before carcinogenesis [19,20]. Additionally, the expression of lamin A/C is impaired by the expression of oncogenic HPV proteins [21].

We have investigated the expression of GATA6 and lamin A/C in human cervical uterine smears (CUS) ahead of the cell morphological alteration with the aim to expose molecular biomarkers for the prevention of cervical cancer ahead of cervical lesions visualized by colposcopy and cytopathology. Our prognostic method could contribute to the prevention of cervical cancer independently of the status in HPV.

2. Material and Methods

2.1. Reagents

Tissue culture flasks, media, trypsin and 100X antibiotic are purchased from Fisher Scientific Inc. (Springfield, NJ, USA). Transfection reagents were purchased from Invitrogen (Carlsbad, CA, USA). Custom primary antibody anti-GATA6 (rabbit IgG) is used for immunoblotting, immunofluorescence and immunohistochemistry [6,7,14].

For immunoblotting we used RIPA buffer (Santa-Cruz biotechnology (CA, USA), protease cocktail inhibitors (Sigma-Aldrich USA); primary antibodies against lamin A/C rabbit IgG and mouse IgG (Santa Cruz biotechnology Inc, Santa Cruz, CA), peroxidase-conjugated secondary antibody anti-rabbit or anti-mouse (Bio-Rad, USA) and Super Signal West Dura Extended Duration Substrate (Fisher Scientific, USA). For immunofluorescence analysis, we used secondary antibodies anti-rabbit or anti-mouse conjugated to Alexa Fluor 488, Alexa Fluor 596 and nuclear counter staining dye DAPI, (Invitrogen, USA).

2.2. Cell Culture

We used primary Human ovarian surface epithelial (HOSE) as control for the expression of GATA6 and lamin A/C. HOSE cells were given by Dr. Andrew Godwin (Fox Chase Cancer Center). HOSE cells were cultured in media containing 6 g/l of HEPES, 15% FBS, 1X penicillin/streptomycin and insulin. All cells were incubated at 37^0C in a humidified atmosphere of 5% CO2 [6,7]. Cervical cancer (Hela) cell line and endometriosis cancer (ECC1) cell lines have been given by Dr. Xiang-xi Xu (Sylvester Cancer Center, University of Miami, USA). All cancer cells were cultured in RPMI media (cell growth, USA).

2.3. Immunofluorescence Microscopy

Briefly, cells were seeded in 4 well chambered slides for 24h. Cells were washed twice with sterile ice-cold-phosphate buffered saline (PBS) at room temperature, fixed with 4% paraformaldehyde for 15 minutes and incubated with 0.5% triton X-100 for 5 minutes to permeabilize cell membrane. Then, cells were washed three times with PBS, blocked with 3% BSA in PBS containing 0.1% tween-20 for 30 minutes, and incubated for 1 hours at 37°C with primary antibodies (1/200 dilution for anti-lamin A, 1/5000 dilution for anti GATA6) in 1% BSA/PBS containing 0.1% tween-20. For the double staining experiment, a goat anti-lamin A (Santa Cruz biotechnology) and a rabbit anti-GATA6 were used. The revelation of GATA-6 and lamin A was achieved using the secondary fluorescence conjugated antibodies; anti-rabbit-Alexa Fluor 488-conjugated (green fluorescence) and anti-goat-Alexa Fluor 594-conjugated (red fluorescence) secondary antibodies were used or vis-versa. Cells were washed three times with PBS, then slides were mounted and sealed in anti-Fade reagent containing 100mM of n-propyl gallate (pH 7.4), 90% glycerol in PBS. The immunofluorescence staining was visualized with 63x or 100x oil objective lens attached to a Nikon Eclipse TE 300 microscope linked to a Roper Scientific photometrics 12-bit range Camera. Image acquisition was done using Meta imaging series (MetaVue) software. Images were merged using MetaVue software [7].

2.4. Immunohistochemistry (IHC)

Archived cervical cancer tissue arrays were from Fox Chase Cancer Center tissue Bank. The method used for IHC analysis was previously described [6,7,8,9]. For GATA6 and lamin A staining, rabbit anti-lamin A and rabbit anti-GATA6 were used conferring to the protocol previously described [6,7,8,9]. GATA6 and lamin A staining were observed with 40X dry objective lens on Nikon Eclipse TE 300 microscope linked to a Roper Scientific Photometrics 12-bit range Camera. Image acquisition was done using Meta imaging series (MetaVue) software.

2.5. Population Investigated

Forty women aged from 20 to 45 years, consulting for a routine gynecological examination in Mènontin hospital located in Cotonou (Benin) were considered for this investigation. Signed informed consents were obtained from all 40 women in this study before cervical uterine smears (CUS) were collected. Among these women we have the hospital personnel, women coming for gynecological check-up after 3 months postpartum and young women who desire to participate to the birth control program of Mènontin hospital.

-The inclusion criteria are women over 20 years old who are already sexually active and consent freely to participate to the study.

- The exclusion criteria: women in menstruation or in first-second month post-partum, women in menstruation or bleeding in the vaginal tract and women under treatment with anti-retroviral drugs.

Due to the high cost of the colposcopic examination the gynecologist made visual inspection of vaginal tract and the uterine-cervix junction but did not take pictures at the beginning of the study. The gynecologist reported 40 women without cervical lesions and 1 woman with cervical lesions at the beginning of the study which is not included. The collection of samples was carried out in the

gynecological service under the supervision of a gynecologist. This study was approved by the Research Ethic Committee of the Institute of Biomedical Science and Application (ISBA), and by the Ministry of Health in BENIN [10]. The procedures followed for this research protocol and sample collection were in accordance with the ethical standards of our institutional and national committees on human experimentation, and in accordance with the Helsinki Declaration.

2.6. Sample Collection

Before the collection of CUS sample, patient was set to be in lithothomy position on the gynecological table. Sample collection was carried out with a disposable sterile speculum introduced in the vagina, followed by an introduction of disposable cytobrush through the speculum to reach the cervical-uterine junction where cells were collected by rotating the cytobrush clockwise twice. To retrieve cells, the brush was released in a 50 ml tube containing 5 ml of ice cold PBS and 50 µl proteases inhibitor. Tubes were kept on ice and delivered within an hour to the Unit of Biochemistry and Molecular Biology (UBBM) and more specifically to the research team of Molecular Biomarkers in Cancer and Nutrition (BMCN), at the Institute of Biomedical Sciences and Applications (ISBA) in Cotonou (Benin) for processing.

2.7. Cell Processing

All tubes were centrifuged to collect cell pellets which were washed again once with ice cold PBS before splitting in 3 eppendorf tubes for: (i) protein analysis by western blotting; (ii) DNA extraction for future investigation and (iii) smearing on glass slide for cell morphology analysis. The majority of collected cells were squamous epithelial cells that will be lysed for protein extraction. The cell pellet was resuspended in 200 µl of RIPA lysis buffer containing 0.5% protease inhibitor cocktail, and kept on ice for 30 min with vigorous agitation every 5 min. An aliquot of cell lysate (2 µl) was used to measure protein concentration with Bio-Rad Dc protein assay kit according to the manufacturer protocol. The remaining of cell lysate was completed with 50 µl of 5x SDS sample buffer and boiled for 5 minutes. Protein samples were kept at -20°C until needed for immunoblotting.

2.8. Western Blot Analysis

Cell lysate was loaded on 4-12% SDS-polyacrylamide gels an run at 100 volts for 2 hours in Tris-Glycin buffer. Proteins were transferred from the SDS-polyacrylamide gels onto nitrocellulose membranes with tris-glycin transfer buffer (100 volts for 1h). The membranes were blocked with 5% milk in 1X TBS and 0.1% Tween-20 (TBST) for 30 minutes at room temperature and incubated with primary antibodies (anti-GATA6, anti-lamin A/C or anti-actin prepared in 1% milk/TBST) for 1 hour at room temperature with gentle agitation. The membranes were washed 4 times for 10 minutes with TBST before incubation with HRP-conjugated secondary antibodies in 1% milk/TBST for 1 hour at room temperature with gentle agitation. Membranes were then washed again 4 times for 15 minutes with TBST before incubation in Super Signal West Dura Extended Duration Substrate for 3 minutes followed by exposure to x-ray film for the revelation of GATA6, lamin A/C or actin [10].

2.9. Incidence of GATA6 and Lamin A Deficiencies

To evaluate the incidence of GATA6 and or lamin A/C deficiency in CUS cells, we attribute number 3 to western blot results with normal expression of GATA6 or lamin A/C, number 2 if the expression is weak and number 0 if there is no expression.

2.10. Video Colposcopy

Two years after the first prognosis, the women were recalled in clinic to perform colposcopy but only fraction of them (n=15) who felt that they have a risk of developing cervical cancer due to the prognostic of the deficiency in GATA6 and lamin A/C came freely for the two-year follow-up. A Video Colposcope Digital Imaging System driven by Multiline preview pic software is used for viewing and documenting colposcopy of women pre-diagnosed with the risk of developing cervical neoplasia. Cervical lesions appeared white when stained with acetic acid (visual inspection with acetic acid, VIAA+) and yellow when stained with Lugol's iodine solution (visual inspection with Lugol's solution, VILI+).

3. Results

3.1. Immunofluorescence Showing Localization of GATA6 and Lamin A/C in Epithelial Cells

GATA6 and lamin A/C are generally present in normal epithelial cells and absent in undifferentiated cells or transformed cells. Here in we validated the localization of both GATA6 and lamin A in normal human ovarian surface epithelial cells (HOSE). The presence of GATA6 and lamin A in HOSE was revealed by immunofluorescence. GATA6 was localized mostly in the nucleoplasm (red) while lamin A was localized around the nuclear envelope and in the nucleoplasm (green) as represented in Figure 1. Image is taken with 100X objective. The localization of GATA6 and lamin A is similar in all differentiated epithelial cells. In neoplastic cell this organization is lost.

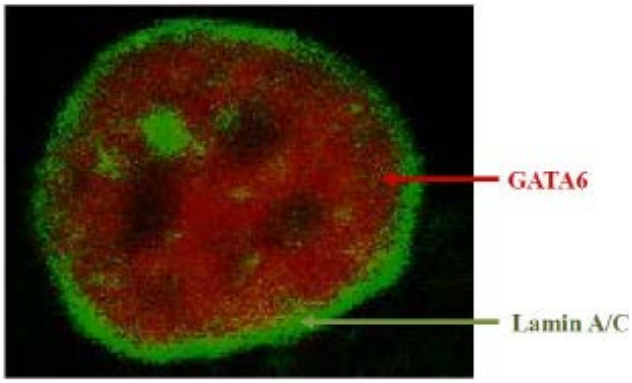

Figure 1. Immunofluorescence showing GATA6 and lamin A. The GATA6 protein is stained in red with secondary anti rabbit-AlexaFluor 546 and lamin A protein is stained in green with secondary anti goat-Alexa Fluor 488. With fluorescence microscopy GATA6 is observed in the nucleoplasm while lamin A is around the nuclear envelope and in the nucleoplasm. Image is taken with 100X objective

3.2. Immunohistochemistry Showing the Absence of GATA6 and /or Lamin A in Cervical Neoplastic Tissues

The Immunohistochemistry (IHC) was performed on archived cervical tissues at Fox Chase Cancer Center in USA. IHC staining of cervical neoplastic tissues and neighboring normal tissues, shows that GATA6 and lamin A are expressed in the nucleus of normal cervical squamous epithelial cells (Figure 2-A). At the earlier stage of cervical intra-neoplasia (CIN-1), GATA6 is totally silenced while lamin A is weak or absent from the nucleoplasm of the majority of epithelial cells (Figure 2-B). The fading of GATA6 disorganized the cervical squamous epithelium and epithelial cells migrated in the stromal region with an anarchic expression of lamin A (Figure 2-B). In a more advanced stage of cervical lesions (CIN-2) both GATA6 and lamin A are absent from the squamous cells (Figure 3-C). Thus the hierarchical expression of GATA6 and lamin A in normal epithelial cells is lost on the pathways to cervical cancer. The brown staining outside the epithelial cells (Figure 2-C) is background. All pictures are taken with a 40X objective.

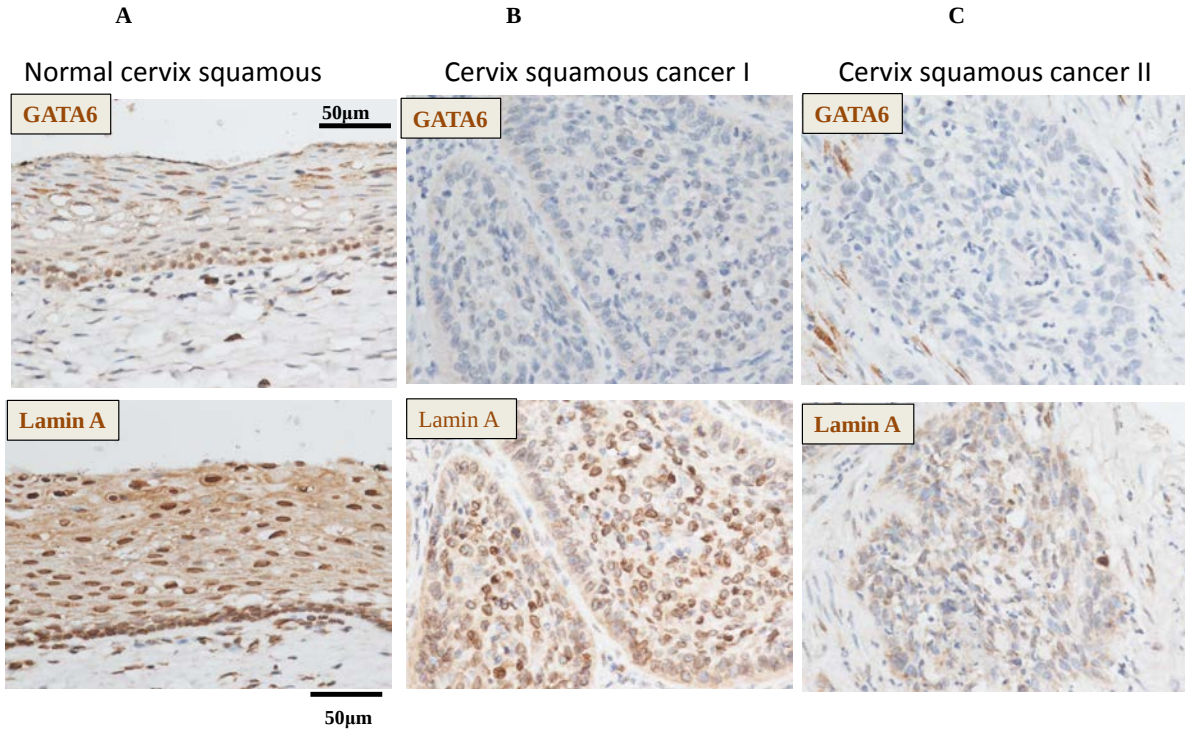

Figure 2. Immunohistochemistry showing that GATA6 and lamin A are expressed in normal squamous epithelial cells of the uterine cervix (A). At earlier stage of cervical intraneoplasia (CIN-1), the silencing of GATA6 is prior to the silencing of lamin A (B). The absence of both GATA6 and lamin A is noticed in the advanced stage of cervical neoplasia CIN-2 (C). Pictures are taken with 40X objective

3.3. Western Blots Showing the Loss of GATA6 and lamin A/C in cervical Squamous Epithelial Cells Collected from CUS.

To verify the expression of GATA6 and lamin A/C in living and seemingly healthy individuals, we lysed cervical surface epithelial cells derived from CUS samples and processed them immediately for western blotting. An example of western blot is shown in Figure 3-A. In sample A1, GATA6 and lamin A/C are normally expressed. In samples C1 and C3 GATA6 is deficient while lamin A/C is expressed. In samples C2 both GATA6 and lamin A/C are deficient. The expressions of GATA6 and lamin A/C are analyzed in CUS of all 40 women selected at the hospital Mènontin in Benin for this study. The results showed that GATA6 is present in 17/40 women (42%), is decreased in 11/40 (28%) and totally absent in 12/40 women (30%). Lamin A/C is present in 25/40 women (62%), low in 10/40 women (25%) and absent in 5/40 women (13%) as shown by histogram in Figure 3-B. Women who are lacking GATA6 and lamin A/C (13%) have a risk to develop cervical intraepithelial neoplasia in the future (Figure 3-B).

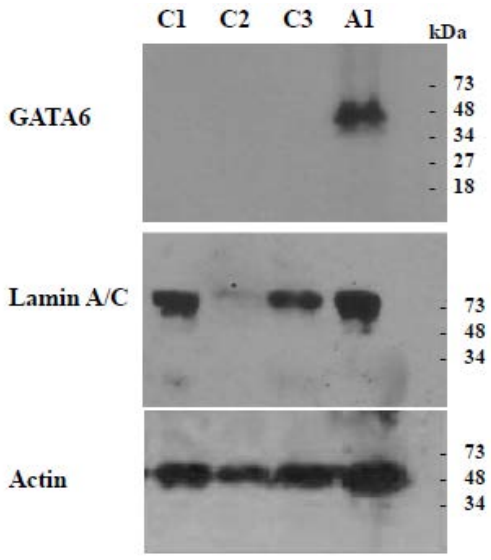

Figure 3-A. Western blot showing simultaneously the expression of GATA6 and lamin A/C in CUS. Normal expression of GATA6 and lamin A/C is observed in sample A1. In samples C1 and C3 GATA6 is absent but lamin A/C is present but low. Sample C2 has no GATA6 or lamin A/C

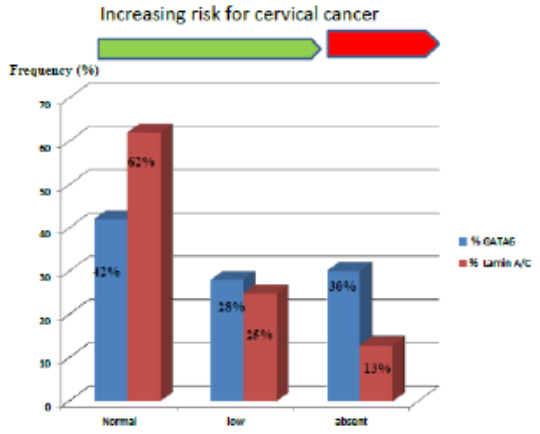

Figure 3-B. GATA6 and lamin A/C have differential expression profile. Both GATA6 and lamin A/C are lost in 13 % of CUS

3.4. Colposcopic Follow-up of Women Pre-diagnosed with the Risk of Developing Cervical Neoplasia

After two-year follow-up period, we have recalled the 40 women without cervical lesions at the beginning of the study, but the majority of them did not come. However, these women have routine gynecological examination and the gynecologist has not reported any abnormalities so far. Only fraction of the women with low expression or deficiency of GATA6 and/or lamin A/C at the beginning of the study (n=15) have agreed for the 2 year follow-up due to the prognosis that they might have a risk to develop cervical neoplasia. Thus, fifteen women came freely for the colposcopic examination of the lower vaginal tract including uterine cervix to determine whether the test results of colposcopy is positive or negative for possible precancerous or cancerous lesions. The colposcopic examination endorsed the observation of squamous intraepithelial lesions using magnification and illumination after applying dilute acetic acid followed by Lugol's iodine solution.

The result shows that 3 /15 females (20%) with the absence of GATA6 and/or lamin A/C have developed a squamous cervical intraepithelial neoplasia. An example of colpophotograph of a woman with squamous intraepithelial lesion is shown (Figure 4). This woman was pre-diagnosed two years ahead with the risk of developing cervical neoplasia based on the absence of GATA6 and lamin A/C but is tested negative for oncogenic HPV. Cervical lesions appeared white when stained acetic acid VIAA+ as shown in Figure 4-A and yellow when stained with Lugol's iodine solution VILI+ as shown in Figure 4-B; the cervical lesions are indicated by black arrows. The genotype of HPV was done for all 40 samples but the data will be presented in different manuscript.

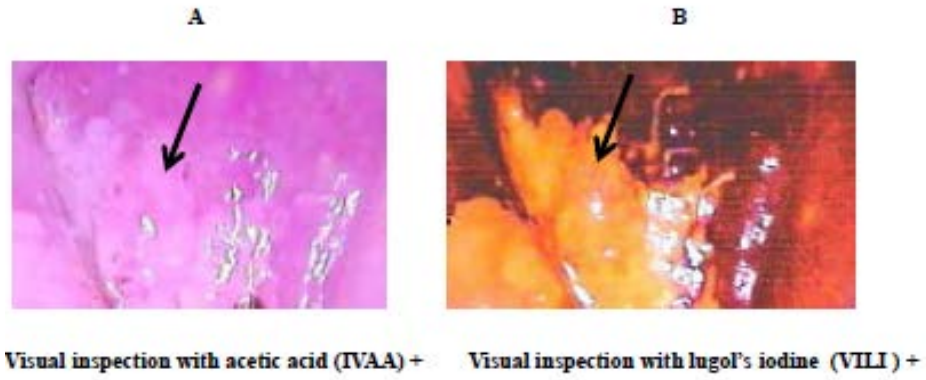

Figure 4. Colpophotograph of a woman with squamous intraepithelial lesions. This woman was pre-diagnosed two year ahead with the risk of developing cervical neoplasia based on the absence of GATA6 and lamin A/C. Cervical lesions appeared white when stained acetic acid (A) and yellow when stained with Lugol's iodine solution (B) as indicted by black arrows

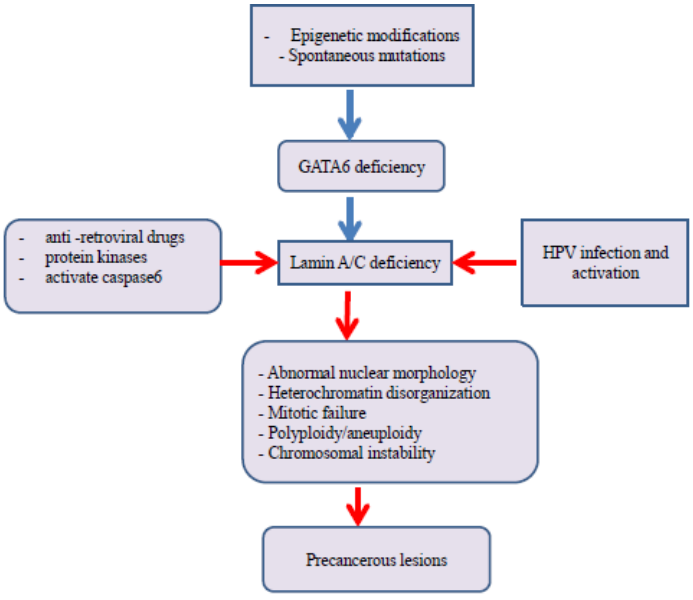

Figure 5. Schema showing the relation between deficiencies of GATA6 and/or lamin A/C leading to precancerous lesions

3.5. Model Showing the Deficiency of GATA6 and/or Lamin A/C in the Initiation and Progression of Cervical Cancer

Epigenetic modifications and especially histone deacetylation is involved in tissue specific deficiency of GATA6, while viral infections, anti-retroviral drugs and deficiency in GATA6 are responsible for the deficiency of lamin A/C (Figure 5). Overall the deficiency of lamin A/C will lead to the alteration in cell division with development of transformed cells and neoplasia. Although the deficiency of GATA6 can reduce lamin A/C, other factors can reduce directly lamin A/C independently of GATA6. All factors that are involved in the deficiency of lamin A/C will initiate cell transformation and neoplasia (Figure 5).

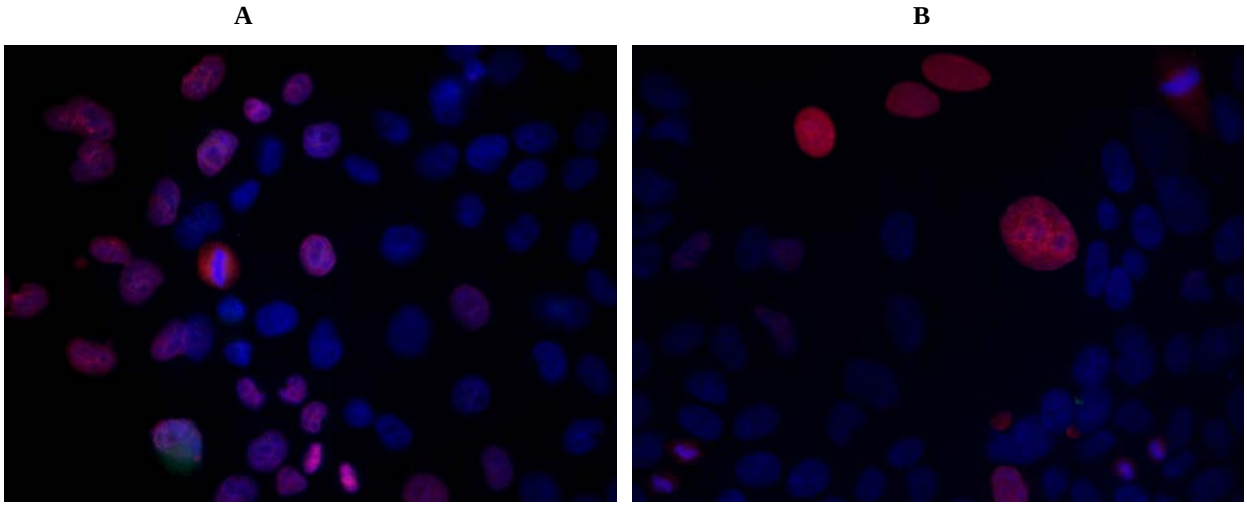

Figure 6. Immunofluorescence showing (A) remaining of GATA6 (green) and lamin A/C (red) expression in cervical carcinoma cell line Hela (A) and (B) in endometriosis carcinoma ECC1 cell line. The absence of GATA6 and lamin A/C is observed in the majority of cervical cancer cell line Hela and endometriosis cancer cell line ECC1. GATA6 is stained with Alexa Fluor 488 (green) while lamin A/C is stained with Alexa Fluor 555 (red) and the nuclear is counterstained with DAPI (blue)

3.6. The Deficiency of GATA6 and Lamin A is Observed in the Majority of Cancer Cells Lines

We have investigated the expression of GATA6 and lamin A/C in several established cell lines including cervical cancer cells (Hela) and endometriosis cancer cells (ECC1). The majority of cancer cell lines do not express GATA6 or lamin A/C. GATA6 is stained with alexafluor 488 (green) and lamin A/C is stained with Alexafluor 555 (red). The nuclear counter stain is carried out with DAPI (blue). GATA6 is totally lost while few subpopulations of cells have lamin A/C (Figure 6). Most cancer cells have abnormal nuclear morphology (Figure 6).

4. Discussion

GATA6 and lamin A/C are present in normal epithelial cells including cervical epithelium (Figure 1, Figure 2). The absence of GATA6 and lamin A/C were previously reported in ovarian cancer cell lines and tissues [6,7,8,9,22]. Herein, we verified that the absence of GATA6 and/or lamin A/C existed prior to the development of cervical neoplasia (Figure 2, Figure 3). The implication of GATA6 and lamin A/C in early carcinogenesis was extensively investigated in ovarian cancer [6,7,8,9,22] but not in cervical-uterine carcinogenesis [10]. It was shown that knockdown of laminA/C with shRNA in cervical cells, initiated abnormal nuclear morphology and heterochromatin disorganization which are hallmarks for cancer [25]. Our previous studies verified that the absence of GATA6 and/or lamin A/C impaired nuclear function leading to failure in cell cycle progression and all the hallmarks of cancer cells including abnormal nuclear shape and size, abnormal DNA replication, polyploidy and aneuploidy [5,7,8,24,26].

The consequence of the total loss of GATA6 and lamin A/C proteins in adults prior to carcinogenesis were poorly documented especially in cervical cancer. The total loss of GATA6 is observed in 30% of women while the total loss of lamin A/C is observed in 13% of women. In all cases the loss of GATA6 is accompanied by a subdual or absence of lamin A/C excepted for one case tested with triple oncogenic HPV (HPV-42, -69,-58). In this case GATA6 was weakly present while lamin A/C was totally absent at the beginning of the study but GATA6 and lamin A/C were totally lost two-years later at the check-up when the lesions were perceptible. This exceptional case could be due to the interference of HPV proteins (E5) with the expression of lamin A/C [21]. The expression of lamin A/C could also be inhibited by HIV protease inhibitor [4,21,27].

Nevertheless, it was shown that the absence of lamin A/C generated mitotic failure, polyploidy and chromosomal instability all of which are hallmarks of cancer cells [7,8,9]. The loss of GATA6 was previously reported as biomarker for cervical cancer prevention but the molecular perturbation underlying it was not fully reported [10]. In this manuscript we reported that the loss of GATA6 was upstream to the loss of lamin A/C except for one case diagnosed with triple oncogenic HPV infection.

The loss of GATA6 in cancer was attributed to histone deacetylation [22,28], while the loss of lamin A and

connected proteins was attributed to protein degradation [7,8]. In most cancer cell lines the down-regulated GATA6 can be restored with histone deacetylase inhibitors [22,28]. The loss of function of GATA6 occurred also with the delocalization of GATA6 protein in the cytoplasm [5,22]. With this investigation *in vitro* and *in vivo* we were able to show that the loss of GATA6 and/or lamin A/C existed prior to the molecular perturbations leading to cancer. In previous studies we were able to reproduce the hallmarks of cancer cells by either disrupting the expression of GATA6 or lamin A [7,8,9].

Both lamin A and GATA6 were makers of differentiated cells and it was predictable that they were missing in cervical squamous epithelial cells prior to cell dedifferentiation and carcinogenesis (Figure 2, Figure 3). From our data it appears that the absence of GATA6 and/or lamin A has significant impact on cell differentiated state, nuclear architecture and epithelium organization [7,8]. The importance of GATA6 and lamin A and other nuclear envelope proteins in gene silencing and chromosomal stability should be further investigated for the prevention of cervical cancer [7,8,24,26]. Taken together it seemed that the epigenetic down-regulation of GATA6 triggered the degradation of lamin A/C to create cells with abnormal nuclear morphology and DNA content (polyploidy/aneuploidy) all of which are signature of cancer cells [22]. Two years after our prognosis for the risk of cervical cancer 3/15 women pre-diagnosed with total loss of lamin A/C have developed cervical neoplasia identified with colposcopy visualization (Figure 4). Tumors deficient in lamin A were reported to be hyperproliferative [29]. We were able to restore the expression of GATA6 and lamin A/C in ovarian and endometrial cancer cells with histone deacetylase inhibitor trichostatin A (TSA) also known as anti-cancer drug [30]. Data will be shown in different manuscript.

In summary, the loss of GATA6 disorganized the cervical squamous epithelium leading to the loss of lamin A/C associated with cell dedifferentiation (Figure 2). Cell dedifferentiation was reported to be hallmark for cancers [5,6,7,8]. Therefore the deficient status of GATA6 and/or lamin A/C in cervical squamous epithelium could be a suitable biomarker for the prognosis of cervical neoplasia. A premature prognosis will help to prevent cervical cancer by promoting cryotherapy, hysterectomy or drug therapy.

5. Conclusion

The results we gathered from previous and recent investigations verified that the absence of GATA6 and/or lamin A/C initiated the hallmark of cancer cells. Our data also pointed out that the absence of GATA-6 and/or lamin A/C in CUS are upstream most of nuclear morphological abnormalities associated with cervical carcinogenesis. Thus, these preliminary results suggests that the deficient expression of GATA6 and/or lamin A/C could be used as biomarkers to identify women at risk of developing cervical lesions. This novel approach to prevent cervical neoplasia should be further taken into consideration and the study will be further extended.

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Statement of Competing Interests

This manuscript summarized a collaboration research work between different institutions in USA and Africa. The authors have no competing interests.

List of Abbreviations

CUS: cervical uterine smears
HOSE: Human Ovarian Surface Epithelial cells
PBS: phosphate Buffered Saline
SDS: sodium dodecyl sulfate
RIPA: Radioimmunoprecipitation assay buffer

References

[1] Louie, K.S., Sanjose, S. and Mayaud P. Epidemiology and prevention of human papillomavirus and cervical cancer in sub-Saharan Africa: a comprehensive review. *Trop Med Int Health*, 14, 1287-1302, Oct 2009.

[2] Bello, F.A., Enaboor, O.O. and Adewole, I.F. Human papilloma virus vaccination for control of cervical cancer: a challenge for developing countries. *Afr J Reprod Health*, 15, 25-30. Mar 2011.

[3] Mitchell, S.M., Sekikubo, M., Biryabarema, C., Byamugisha, J.J., Steinberg, M., Jeronimo, J., Money, D.M., Christilaw, J. and Ogilvie, G.S. Factors associated with high-risk HPV positivity in a low-resource setting in sub-Saharan Africa. *Am J Obstet Gynecol*, 210 (1), 81.e1-7, Aug 2014.

[4] Jaquet, A., Horo, A., Ekouevi, D.K., Toure, B., Coffie, P.A., Effi, B., Lenaud, S., Messou, E., Minga, A., Sasco, A.J. and Dabis, F. Risk Factors for Cervical Intraepithelial Neoplasia in HIV Infected Women on Antiretroviral Treatment in Côte d'Ivoire, West Africa. *PLoS One*, 9 (3), e90625, Mar 2014.

[5] Capo-chichi, C.D., Roland, I.H., Vanderveer, L., Bao, R., Yamagata, T., Hirai, H., Cohen, C., Hamilton, T.C., Godwin, A.K. and Xu, X.X. Anomalous expression of epithelial differentiation-determining GATA factors in ovarian tumorigenesis. *Cancer Res*, 63(16), 4967-4977, Aug 2003.

[6] Cai, K.Q., Caslini, C., Capo-chichi, C.D., Slater, C., Smith, E.R., Wu, H., Klein-Szanto, A.J., Godwin, A.K. and Xu, X.X. Loss of GATA4 and GATA6 expression specifies ovarian cancer histological subtypes and precedes neoplastic transformation of ovarian surface epithelia. *PLoS One*, 4(7):e6454, Jul 2009.

[7] Capo-Chichi, C.D., Cai, K.Q., Testa, J.R., Godwin, A.K. and Xu, X.X. Loss of GATA6 Leads to Nuclear Deformation and Aneuploidy in Ovarian Cancer. *Mol. Cell Biol*, 29(17), 4766-4777, Sep 2009.

[8] Capo-chichi, C.D., Cai, K.Q., Simpkins, F., Ganjei-Azar, P., Godwin, A.K. and Xu, X.X. Nuclear envelope structural defects cause chromosomal numerical instability and aneuploidy in ovarian cancer. *BMC Med*, 9, 28, Mar 2011.

[9] Capo-Chichi, C.D., Cai, K.Q., Smedberg, J., Ganjei-Azar, P., Godwin, A.K. and Xu, X.X. Loss of A-type lamin expression compromises nuclear envelope integrity in breast cancer. *Chin J Cancer*, (6):415-425, Jun 2011.

[10] Capo-chichi, C.D., Alladagbin, J.D., Brun, L., Aguida, B., Agossou, K.V., Anagbla, T., Salmane, A., Xu, X.X. and Sanni, A. Deficiency of GATA6 as a Molecular Tool to Assess the Risk for Cervical Cancer. *American Journal of Biomedical Research*, 2 (1), 1-6, Jan 2014.

[11] Capo-chichi, C.D., Rula, M.E., Smedberg, J.L., Vanderveer, L., Parmacek, M.S., Morrisey, E.E., Godwin, A.K. and Xu, X.X. Perception of differentiation cues by GATA factors in primitive endoderm lineage determination of mouse embryonic stem cells. *Dev. Biol*, 286(2), 574-586, Oct 2005.

[12] Capo-chichi, C.D., Smedberg, J.L., Rula, M., Nicolas, E., Yeung, A.T., Adamo, R.F., Frolov, A., Godwin, A.K. and Xu, X.X. Alteration of Differentiation Potentials by Modulating GATA Transcription Factors in Murine Embryonic Stem Cells. *Stem Cells Int*, 11, 2010:602068, May 2010.

[13] Caruso, R.A., Fedele, F., Consolo, P., Luigiano, C., Venuti, A. and Cavallari, V. Abnormal nuclear structures (micronuclei, nucleoplasmic bridges, and nuclear buds) in a pleomorphic giant cell carcinoma of the stomach. *Ultrastruct Pathol*, 32(1), 11-15. Jan-Feb 2008.

[14] Butler, J.T., Hall, L.L., Smith, K.P. and Lawrence, J.B. Changing nuclear landscape and unique PML structures during early epigenetic transitions of human embryonic stem cells. *J Cell Biochem*, 107(4), 609-621, Jul 2009.

[15] Capo-chichi CD, Smith ER, Rosario S, Ganjei-Azar P , Xu XX. Nuclear envelope defects in epithelial ovarian cancer. *Am. J. Clin. Exp. Obstet. Gynecol*, 1(1), 17-30, Dec 2013.

[16] Corso, C., Parry, E.M., Faragher, R.G., Seager, A., Green, M.H. and Parry, J.M. Molecular cytogenetic insights into the ageing syndrome Hutchinson-Gilford Progeria (HGPS). *Cytogenet Genome Res*, 111(1), 27-33, 2005.

[17] Capell, B.C. and Collins, F.S. Human laminopathies: nuclei gone genetically awry. *Nat Rev Genet*, 7(12), 940-952, Dec 2006. Review.

[18] Ward, K.K, Berenson, A.B., and Breitkopf, C.R. Passive smoke exposure and abnormal cervical cytology in a predominantly Hispanic population. Am J Obstet Gynecol, 204(3):213 e1-6, Mar 2011.

[19] Lazarenko, L.M., Nikitina, O.E., Nikitin, E.V., Demchenko, O.M., Kovtonyuk, G.V., Ganova, L.O., Bubnov, R.V., Shevchuk, V.O., Nastradina, N.M., Viktoria, V. V. and Spivak, M.Y. Development of biomarker panel to predict, prevent and create treatments tailored to the persons with human papillomavirus-induced cervical precancerous lesions. *EPMA Journal*, 5(1), Jan 2014.

[20] Adam ML, Pini C, Túlio S, Cantalice JC, Torres RA, Correia MT. Assessment of the Association Between Micronuclei and the Degree of Uterine Lesions and Viral Load in Women with Human Papillomavirus. Cancer Genomics Proteomics, 12(2):67-71. Mar 2015.

[21] Kivi, N., Greco, D., Auvinen, P., Auvinen, E. Genes involved in cell adhesion, cell motility and mitogenic signaling are altered due to HPV 16 E5 protein expression. Oncogene, 27(18):2532-2541. Apr 2008, Epub Nov 2007.

[22] Caslini, C., Capo-chichi, C.D., Roland , I.H., Nicolas, E., Yeung, A.T., and Xu XX. Histone modifications silence the GATA transcription factor genes in ovarian cancer. Oncogene, 25(39), 5446-5461, Aug 2006.

[23] Kosho, T., Takahashi, J., Momose, T., Nakamura, A., Sakurai, A., Wada, T., Yoshida, K., Wakui, K., Suzuki, T., Kasuga, K., Nishimura, G., Kato, H. and Fukushima, Y. Mandibuloacral dysplasia and a novel LMNA mutation in a woman with severe progressive skeletal changes. *Am J Med Genet A*, 143(21), 2598-2603, Nov 2007.

[24] Somech, R., Shaklai, S., Amariglio, N., Rechavi, G. and Simon, A.J. Nuclear envelopathies--raising the nuclear veil. *Pediatr Res*, 57(5 Pt 2), 8R-15R, May 2005, Review.

[25] Chaturvedi, P., Khanna, R., Parnaik,V.K. Ubiquitin ligase RNF123 Mediates Degradation of Heterochromatin Protein 1α and β in Lamin A/C Knock-Down Cells. *Plos One*, 7 (10) e47558, Oct 2012.

[26] Shaklai, S., Amariglio, N., Rechav,i G. and Simon, A.J. Gene silencing at the nuclear periphery. *FEBS J*, 274(6), 1383-1392, Mar 2007. Review.

[27] Hudon, S.E., Coffinier, C., Michaelis, S., Fong, L.G., Stephen, G., Young, S.G and Hrycyna, C,A. HIV-Protease Inhibitors Block the Enzymatic Activity of Purified Ste24p. *Biochem Biophys Res Commun*, 374(2), 365–368. Sep, 2008.

[28] Zhou, J., Hernandez, G., Tu, S.W., Scholes, J., Chen, H., Tseng, C.P. and Hsieh, J.T. Synergistic induction of DOC-2/DAB2 gene expression in transitional cell carcinoma in the presence of GATA6 and histone deacetylase inhibitor. *Cancer Res*, 65(14), 6089-96, Jul 2005.

[29] Venables, R.S., McLean, S., Luny, D., Moteleb, E., Morley, S. and Quinlan, R.A, Lane EB, Hutchison CJ. Expression of individual lamins in basal cell carcinomas of the skin. *Br J Cancer*, 84(4),512-519, Feb 2001.

[30] Galiová, G., Bártová, E., Raska, I., Krejcí, J. and Kozubek, S. Chromatin changes induced by lamin A/C deficiency and the histone deacetylase inhibitor trichostatin A. *Eur J Cell Biol*, 87(5):291-303, May 2008.