

Deficiency of GATA6 as a Molecular Tool to Assess the Risk for Cervical Cancer

Capo-chichi D. Callinice^{1,*}, Alladagbin D. Jeanne¹, Brun Luc⁴, Aguida Blanche¹, Agossou Komlan Vidéhouéno², Anagbla Toussain³, Salmane Amidou⁴, Xu Xiang-Xi⁵, Sanni Ambaliou¹

¹Faculty of Sciences and technology (FAST) / Laboratory of Biochemistry and molecular biology (LBBM), Institute of Biomedical Sciences and Applications (ISBA) / University Abomey-Calavi (UAC), BENIN

²Gynecology/obstetric and oncology services, CNHU, UAC, Cotonou, BENIN

³Gynecology/obstetric service, Hospital Mènontin, Cotonou, BENIN

⁴Gynecology/obstetric service and CIPEC, CHDU Borgou, Parakou, BENIN

⁵Sylvester Cancer Center/Miller Medical School of Medicine, University of Miami, USA

*Corresponding author: callinice.capochichi@gmail.com

Received December 19, 2013; Revised January 09, 2014; Accepted January 14, 2014

Abstract Introduction: GATA6 is a transcription factor which has role in the induction of cell differentiation genes and the maintenance of the differentiated state of epithelial cells. GATA6 expression is lost in neoplastic ovarian epithelia cells and in ovarian carcinoma leading to abnormal nuclear morphology characteristic of most cancer cells. We investigated the profile of GATA6 in cells collected from cervical-uterine smears (CUS) of women in the gynecologic service of three hospitals in BENIN. **Objective:** To utilize GATA6 as molecular marker for the screening of women at risk of developing cervical carcinomas. **Methods:** CUS were collected from forty (40) women coming for regular checkup (a) at the National University Hospital (CNHU) in Cotonou and (b) from the local hospital of Mènontin (HZ) in Cotonou (south of BENIN); (c) forty others (40) CUS were collected from women coming for treatment against HIV1 in the service of gynecology of the Departmental University Hospital (CHDU) of Borgouin Parakou (north of BENIN). GATA6 was analyzed in cells isolated from 80 CUS totally by the immunoblotting techniques. **Results:** In women from Cotonou, GATA6 was present in 17/40 (42%) CUS, lightly expressed in 10/40 (25%) CUS and totally absent in 13/40 (32.5%) CUS. In the HIV1 infected women under treatment in Parakou, GATA6 was present in 8/40 (20%) CUS, lightly expressed in 13/40 (32.5%) and totally lost in 19/40 (47.5%) CUS. **Conclusion:** Our study showed that the loss of GATA6 in CUS was significantly higher in the population of women infected with HIV1 than in women from regular population in Cotonou. Thus the deficiency in GATA6 expression maybe utilized as diagnostic tools to identify women at risk for developing cervical carcinomas regardless the infectious status before the onset of neoplasia.

Keywords: GATA6, Cervical-Uterine smears, cervical carcinomas

Cite This Article: Capo-chichi D. Callinice, Alladagbin D. Jeanne, Brun Luc, Aguida Blanche, Komlan Agossou Komlan Vidéhouéno, Anagbla Toussain, Salmane Amidou, Xu Xiang-Xi, and Sanni Ambaliou, "Deficiency of GATA6 as a Molecular Tool to Assess the Risk for Cervical Cancer." *American Journal of Biomedical Research* 2, no. 1 (2014): 1-6. doi: 10.12691/ajbr-2-1-1.

1. Introduction

The transcription factor GATA6 is known as a zinc finger protein that binds to consensus sequence 5'-A/T-GATA-A/G-3' at the promoter region of target genes involved in cell differentiation [1,2,3]. Six (06) members of GATA proteins are identified including GATA1, GATA2, GATA3 expressed in hematopoietic cell lineage; and GATA4, GATA5, GATA6 expressed in the endodermic cell lineage [4,5,6].

During embryogenesis GATA6 is involved in endodermic cell differentiation and cell lineage specification [4,5,6]. In adults, GATA6 maintains the differentiated state of epithelial cells [7]. The lack of expression of GATA6 is known to cause the loss of the

expression of target genes, leading to the dedifferentiation of epithelial cells, to chromosomal instability and carcinogenesis [8,9]. The dedifferentiation of epithelial is accompanied by abnormalities in nuclear morphology, chromosomal instability, polyploidy and aneuploidy all of which are bases for epithelium disorganization and carcinoma including ovarian carcinoma [10]. The loss of GATA6 in epithelial cells at the cervical-uterine junction, maybe a biomarker indicator of risk for developing cervical pre-neoplastic or neoplastic lesions.

Cervical cancer is one of the principal cancers causing death among women because its diagnosis is done when it has already progressed and difficult to cure with poor survival rate [11,12]. Cervical carcinomas are caused by numerous factors including viral infection (HPV, HIV), mutation in tumor suppressor genes (P53, Rb), chromosomal aberration, epigenetic modifications [11,12].

Most genes involved in cell cycle regulation and epithelial cell differentiation can be silenced by epigenetic modification giving rise to loss of expression of GATA6, cell dedifferentiation, abnormal cell cycle progression, abnormal nuclear morphology and polyploidy/aneuploidy observed in cancer cells but not in normal cells [8,11,12,13]. Previous studies done by our research team on ovarian cancer had demonstrated that the loss of GATA6 was responsible for the nuclear abnormalities and chromosomal numerical instability leading to cancer [10]. Thus the loss of GATA6 led to all the hallmark of carcinoma and was prior to the initiation of tumor [8,9,10] making the loss of GATA6 a candidate biomarker to assess the risk for the initiation of the hallmark of cancer including cervical cancer.

In 2011 statistical data of Ministry of Public Health in BENIN reported 1408 malignant tumors with 333 gynecological (24%). Based on that report it is becoming urgent to find a biomarker to diagnose transformed cells regardless of viral infection and start prophylactic treatment before the progression toward precancerous or cancerous lesions. The utilization of GATA6 deficiency in CUS as diagnostic tool will decrease women mortality related to cervical cancer.

2. Material and Methods

2.1. Study Ground

Sample treatment and protein analysis were done in the laboratory of Biochemistry and Molecular Biology (LBBM) of the University Abomey-Calavi (UAC) in BENIN and especially in the division of Molecular Biomarker in Cancer and Nutrition (BMCN). LBBM and BMCN are localized in the Institute of Biomedical Sciences and Applications (ISBA) Cotonou, BENIN. Sample collections were done at the National University Hospital of Cotonou (CNHU), at the Local Hospital of Mènontin (HZ Mènontin) in Cotonou and at the Departmental University Hospital of Borgou (CHDU Borgou) at Parakou. This study was approved by the ethical comity of ISBA and by the Ministry of Public Health in BENIN.

2.2. Populations

2.2.1. Inclusion Characteristics

- Forty (40) women over 20 years old sexually active and coming to hospital for regular check-up including hospital personnel. Among forty (40) women recruited in Cotonou, thirteen (13) of them came to CNHU for inflammation of the cervical-uterine junction and twenty seven (27) of them came to HZ Mènontin for child birth control service.

- Forty (40) women over 20 years old sexually active and coming to hospital CHD Borgou to receive treatment against previously diagnosed HIV1 infection. This HIV status was diagnosed by the HIV treatment center (CIPEC) in CHD Borgou in the city of Parakou.

- Informed consent was obtained from each woman before the collection of samples.

2.2.2. Exclusion Characteristics

- Pregnancy
- Menstruation
- Virginity
- Anemia

2.3. Biological Samples and Reagents

Biological samples used were cell lysates from CUS collected from 80 women designated above. All chemical reagents are mostly purchased from Sigma-Aldrich and Bio-Rad. Primary antibody against GATA6 was custom made in rabbit [14], antibody anti β -actin made in mouse was purchased from Sigma-Aldrich (USA). Peroxidase conjugated-Secondary antibodies against rabbit or mouse were purchased from Bio-Rad (USA) and reagent for chemoluminescence revelation of secondary antibody (SuperSignal West Dura Extended Duration Substrate) was purchased from Thermo Scientific (USA). RIPA buffer was purchased from Santa-Cruz Biotechnology (USA).

2.4. Collection, Processing and Storage of CUS Cell Lysates

Disposal cytobrush was used to collect cervical liquid containing epithelial cells. A speculum was introduced into the vagina to help bring in the cytobrush through the cervix. One rotation of the brush clockwise collected the cervical-uterine liquid containing the cells. The brush was deposited in a 5 ml tube containing ice cold Phosphate-Buffered Saline (PBS) kept on ice in a cooler. Tubes containing CUS and PBS were centrifuged at 3000 rpm for 5 min to collect cell pellet and RIPA buffer (200 μ l) was used to lysate the cells. Aliquots of cell lysate were made to use for protein quantification (50 μ l) and for western blotting (150 μ l).

To denature the proteins, 4X of SDS buffer (50 μ l) containing 60 mM of Tris-HCl pH 6,8 ; 2% SDS ; 10% Glycérol; 5% β -mercaptoethanol and 0,01% de bleu de bromophenol, was added to the 150 μ l cell lysate to reach 200 μ l. The cell lysate was boiled at 95°C for 10 min in a dried sand-bath. Denatured cell lysates were stored at -20°C until needed for western blotting according to the protocol previously described [10]. For protein analysis samples were boiled again at 95°C for 10 min and loaded on a 10% polyacrylamide gel. The migration of the gel was done in a Tris-glycine solution. Proteins were transferred on a nitrocellulose membrane with a Tris-glycine-20% methanol solution. The Membrane was blocked with 5% milk-TBST (Tris buffered saline tween-20 solution containing Tris-base 25 mM; NaCl 150 mM; KCl 2 mM; 0.05% tween-20.) for 30 min.

The membrane was then incubated in a primary antibody against GATA6 (prepared in 1% milk-TBST) for 60 min at room temperature.

The membrane was washed 3 times 5 min in TBST solution and then incubated in a secondary peroxidase conjugated antibody anti-rabbit (prepared in 1% milk-TBST) for 60 min at room temperature. The membrane was washed 4 times 5 min in TBST solution before incubation in a 2ml mixture of chemoluminescence reagent "Super Signal West Dura Extended Duration Substrate" for 5 min followed by the exposure of the membrane to an X-ray film and development in a film processor for protein detection. In a second run, the

membrane was stripped and blocked into 5% milk-TBS for 30 min before incubation in a primary antibody anti- β -actinsolution as loading control. Membrane was washed as described above and then incubated in a secondary peroxidase conjugated antibody anti-mouse (prepared in 1% milk-TBST) for 60 min at room temperature. Membrane was washed and incubated in a chemoluminescence reagent and exposed to an X-ray film as specified above.

2.5. Microscopic Observation

Aliquots of cells isolated from CUS were fixed in methanol/PBS at -20°C overnight, sprayed over glass slide and air dried in incubator (55°C) for 3 hours to attach the cells onto the glass slides. The slides were immersed in a solution of hematoxylin for 20 second to stain the nuclei. The slides were then immersed in pure water 3 times 5 min to rinse the cells and let dry over night at room temperature. Mounting medium was added to the cells on the slides and sealed with a cover slip on top of the slide. The nuclear morphology and quantity were observed with a Nikon microscope under 20X objective. Pictures were taken with a Nikon digital camera and processed with Adobe Photoshop software.

2.6. Statistical Analysis

The two data samples were independent and from distinct populations. The non-parametric method, Mann-Whitney-Wilcoxon test was used to compare the

expression of GATA6 in CUS samples collected from women in Cotonou to CUS samples from women in Parakou. For statistical analysis, the CUS with strong expression of GATA6 was attributed with an arbitrary value of 3, the CUS with weak expression of GATA6 was attributed with an arbitrary value of 2, and CUS with no GATA6 expression was attributed with an arbitrary value of 1. The difference between the two groups was considered significant for $p < 0.05$.

3. Results and Discussion

3.1. GATA6 Expression in Cervical-uterine Smears

The results of western blotting with anti-GATA6 antibody showed GATA6 band around 50 KDa. Strong signal showed the expression of GATA6, lighter signal indicated weak expression of GATA6 while the absence of signal indicated the loss of expression of GATA6 (Figure 1). On the western blot membrane showed as example in Figure 1, the expression of GATA6 was noticed in samples 1-54P, 1-56P and 1-59P while no expression of GATA6 was observed for samples 1-55P, 1-57P, 1-58P and 1-60 P. The β -actin bands were used as loading control. Samples 1-55P and 1-57P had few cells in the CUS. The sample numbers were scrambled and could not identify the donors of CUS.

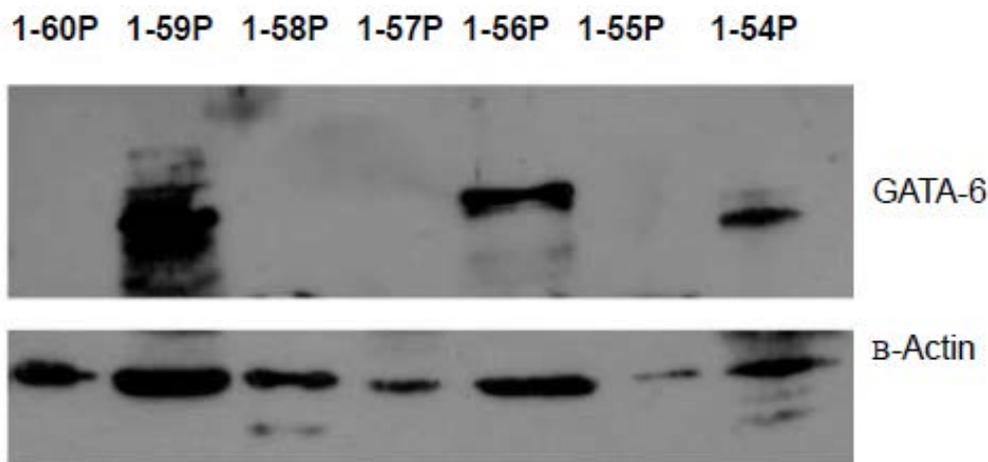


Figure 1. Example of GATA6 expression in some cervical-uterine smear cell lysates. GATA6 was expressed in samples 1-59P, 1-56P, 1-54P and absent in samples 1-55P, 1-57P, 1-58P and 1-60P. β -Actin was used as loading control

Table 1. Summary of GATA6 expression in 80 cervical-uterine smears in BENIN

| Results | Expression of GATA6 | Percentage Cotonou (N=40) | Percentage Parakou (N=40) |
|----------------|---------------------|---------------------------|---------------------------|
| No risk (NR) | Normal+++ [3] | 42.5% (17/40) | 20.0 % (8/40) |
| Low risk (LR) | Weak(+) [2] | 25.0% (10/40) | 32.5 % (13/40) |
| High risk (HR) | Absent(-) [1] | 32.5% (13/40) | 47.5% (19/40) |

N: number of samples, NR : no risk of developing cervical carcinoma.

LR: low risk of developing cervical carcinoma, HR: high risk of developing cervical carcinoma. Presence of GATA6 was represented by (+++) or [3], weak expression of GATA6 was represented by (+) or [2], while total absence of GATA6 was represented by (-) or [1].

All results from the western blots were presented in Table 1 and Table 2, according to the city (Cotonou and Parakou). Presence of GATA6 was represented by (++++) with an arbitrary value of 3, weak expression of GATA6 was represented by [+] with an arbitrary value of 2 while total absence of GATA6 was represented by [-] with an arbitrary value of 1. The details of GATA6 expression in each CUS was shown in Table 1.

Overall, from the protein analysis done in the two populations of 40 samples each (total of 80 samples), we observed the expression of GATA6 in 17/40 (42.5%) samples from hospital in Cotonou (CNHU and HZ Mènontin) and 8/40 (20%) from samples from hospital in Parakou (CHUD Borgou). Weak expression of GATA6 was observed in 10/40 (25%) samples from Cotonou and

in 13/40 (32.5%) samples from Parakou. Total absence of GATA6 was observed in 13/40 (32.5%) samples from Cotonou and 19/40 (47.5%) samples from Parakou (Table 2). The group with HIV infection and receiving antiretroviral treatment (Parakou) had more frequency of GATA6 deficiency compare to the regular population with unknown viral status (Cotonou). In consideration for the inflammation status, the loss of GATA6 was not more frequent in CUS from women with cervical -- uterine inflammation, as 8/13 were expressing GATA6; 4/13 had weak expression of GATA6 and only 1/13 had lost the expression of GATA6 (Table 1).The loss of expression of GATA6 is independent of infectious and inflammatory status (Table 1).

The percentages of CUS with strong, weak and no expression of GATA6 were presented in Table 2. Samples with strong expression of GATA6 were not considered at risk of developing cervical carcinoma (NR), samples with weak expression of GATA6 had low risk of developing cervical carcinoma (LR) while samples with total absence of GATA6 had high risk of developing cervical carcinoma (HR). Thus regular population from Cotonou hospital had fewer women at risk of developing cervical carcinoma than HIV1 infected women from Parakou hospital (32.5% versus 47.5%) respectively.

The presence of GATA6 was a control for the non-dedifferentiation of epithelial cells while the absence of GATA6 was an indicator of cell dedifferentiation and transformation [8,9,10]. Thus the absence of GATA6 identified the woman at risk of developing cervical cancer because they are bearing dedifferentiated and transformed cells in the cervical-uterine epithelium. The risk is stronger in women with HIV1 infection than in the regular population. The difference is significant (p < 0.005).The Figure 2 showed histograms comparing both populations (regular versus HIV1 infected). According to the profile of GATA6, women with HIV1 infection had more risk of developing cervical carcinoma in the future than women in general population. Those women were not tested for HPV infection.

Table 2. GATA6 expression in CUS samples from Cotonou and Parakou

| Sample No Coionou | Results GATA6 Coionou | Sample No Parakou | Results GATA6 Parakou |
|-------------------|-----------------------|-------------------|-----------------------|
| 2-10C | 3 | 1-02P | 3 |
| 2-11C | 3 | 1-11P | 3 |
| 2-18C | 3 | 1-15P | 3 |
| 2-31C* | 3 | 1-16P | 3 |
| 2-35C* | 3 | 1-19P | 3 |
| 2-36C* | 3 | 1-28P | 3 |
| 2-37C* | 3 | 1-59P | 3 |
| 2-38C* | 3 | 1-64P | 3 |
| 2-39C* | 3 | 1-01P | 2 |
| 2-40C* | 3 | 1-03P | 2 |
| 2-41C* | 3 | 1-05P | 2 |
| 2-70C | 3 | 1-08P | 2 |
| 2-75C | 3 | 1-10P | 2 |
| 2-76C | 3 | 1-12P | 2 |
| 2-78C | 3 | 1-17P | 2 |
| 2-80C | 3 | 1-21P | 2 |
| 2-87C | 3 | 1-23P | 2 |
| 2-12C | 2 | 1-56P | 2 |
| 2-139C | 2 | 1-61P | 2 |
| 2-17C | 2 | 1-63P | 2 |
| 2-32C* | 2 | 1-14P | 2 |
| 2-33C* | 2 | 1-4P | 1 |
| 2-42C | 2 | 1-6P | 1 |
| 2-54C* | 2 | 1-7P | 1 |
| 2-72C | 2 | 1-9P | 1 |
| 2-75C | 2 | 1-13P | 1 |
| 2-89C | 2 | 1-18P | 1 |
| 2-137C | 1 | 1-20P | 1 |
| 2-138C | 1 | 1-22P | 1 |
| 2-145C | 1 | 1-24P | 1 |
| 2-16C | 1 | 1-25P | 1 |
| 2-34C* | 1 | 1-26P | 1 |
| 2-69C | 1 | 1-27P | 1 |
| 2-71C | 1 | 1-29P | 1 |
| 2-73C | 1 | 1-30P | 1 |
| 2-82C | 1 | 1-55P | 1 |
| 2-82C | 1 | 1-57P | 1 |
| 2-85C | 1 | 1-58P | 1 |
| 2-86C | 1 | 1-60P | 1 |
| 2-88C | 1 | 1-62P | 1 |

To facilitate the statistical analysis of the data, an arbitrary number of 3 was given for the expression of GATA6; 2 was given for weak expression of GATA6 and 1 was given for the absence of expression of GATA6. The CUS with inflammation were marked with an asterisk *.

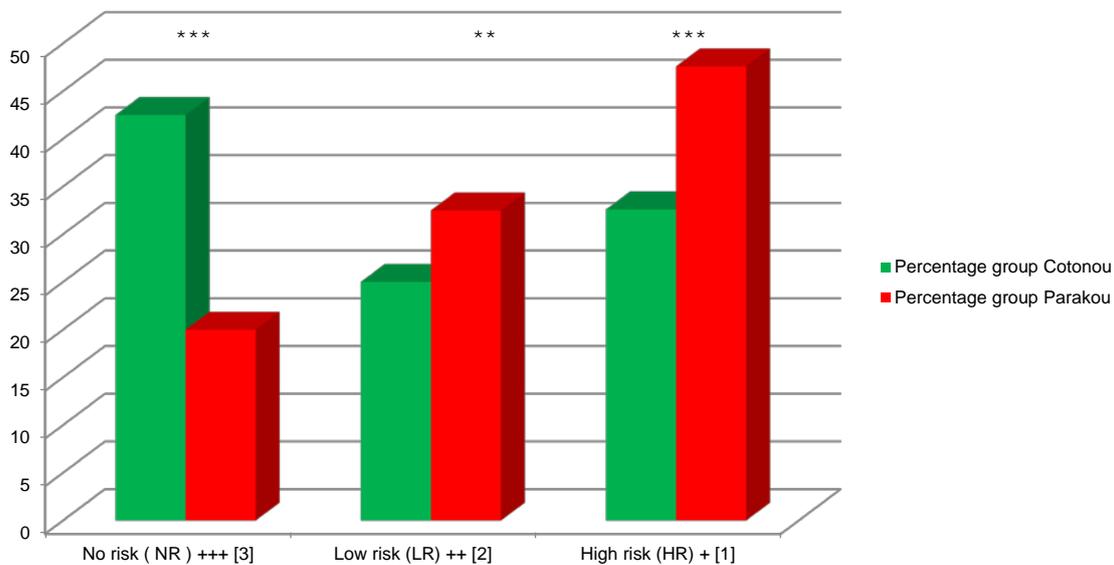


Figure 2. Histograms representing GATA6 expression in regular population of Cotonou versus GATA6 expression in HIV1 infected women from Parakou. Women with aberrant expression of GATA6 were higher in HIV1 infected population (Parakou) than in regular population (Cotonou). NR: no risk, LR: low risk, HR: high risk for cervical cancer. Significant differences were observed for the three categories in both populations. [**] for p<0.05 and [***] for p < 0.001

3.2. Cell Morphology Analysis by Microscopy

The cell nucleus was enlarged and some had multiple nuclei in all CUS samples without GATA6 expression

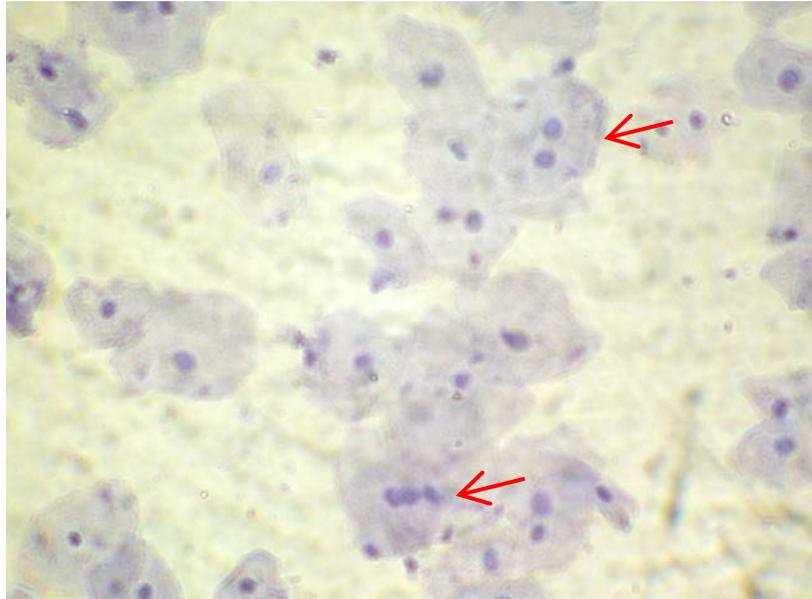


Figure 3. photography representing cell morphology and nuclear abnormality in regular population of Cotonou lacking GATA6. Cells with multiple nuclei were designated by a red arrow

4. Discussion

The transcription factors GATA were important for cell differentiation and organ specification during embryogenesis [4,14,18]. GATA6 expression was induced by embryonic cell aggregation and retinoic acid [4,14]. Lack of expression of GATA6 during embryogenesis led to a disorganized primitive endoderm cells and non-viable embryo [1,14,15]. In ovarian epithelium, the absence of expression of GATA6 induced the loss of differentiated feature of epithelial cells leading to cell dedifferentiation and disorganized epithelium encountered in carcinomas including ovarian carcinomas [8,10,16,17,18].

Cell dedifferentiation is caused by the loss of differentiation protein such as disabled-2 which promoter is stimulated by GATA6 transcription factor [2,19]. Dedifferentiated cells no longer respond to cell growth regulation imposed by the epithelium structure and cooperate with acquired oncogenic stimuli to initiate tumor development as it was reported in ovarian carcinomas [2]. The results of our GATA6 investigation in CUS showed that women with HIV1 infection (47.5%) were more prone to cervical carcinomas than women in regular population (32.5%). Thus the loss of GATA6 put women at risk of developing cervical carcinomas because it will induce all the hallmark of cancer cells as previously reported [8,9,10,17]. Previously research analysis in ovarian tissue biopsies showed that 38% of ovarian tumor tissues had lost GATA6 expression [8,9,10]. Absence of expression of GATA6 led to a loss of epithelial differentiation proteins (Dab2), to cell dedifferentiation, nuclear deformation, abnormal cell cycle, chromosomal instability, polyploidy and aneuploidy all of which were background for tumor initiation [2,8,9,10,17]. According to previous studies we had demonstrated extensively that the loss of GATA6 led to the hallmarks of ovarian

(Figure 3). The presence of multinucleated cells is sign of polyploidy similar to polyploid cells observed in carcinoma cells lacking GATA6 expression [10].

carcinoma including polyploidy [2,8,9,10,17]. In our present study, the lack of GATA6 was associated with enlarged cell nucleus or multiple nuclei in the cell indicating a polyploid caryo type in cells from CUS lacking GATA6.

Taking into consideration the main role played by GATA6 in cell differentiation, its absence in cervical epithelial cells suggested that they were already engaged in transformation pathways that could lead to tumor if no action was taken. Thus, deficiency of GATA6 in CUS cells was a candidate biomarker for screening of women at risk for developing cervical cancer [2,10].

The phenomenon leading to the reduction of GATA6 in adult cells could be due to an epigenetic modification (histone H3, H4 hypoacetylation or methylation at the CpG island of the promoter); or to spontaneous mutations as it had been shown [9,10]. Deficiency in vitamin A could also account for the decrease of expression of GATA6 due to the reduction of the stimulation of its promoter [4,14]. In case of epigenetic modification, the expression of GATA6 can be restored with a combination of histone desacetylase inhibitor trichostatin (TSA) and DNA demethylation agent 5-AZA-cytidine [9,10]. Treatment with vitamin A could help to enhance the expression of GATA6 [2,4,9,14]. Thus the loss of GATA6 could be corrected to prevent progression toward carcinogenesis. We will follow up the women over a period of 5 years to evaluate the progression or not toward cancer development.

5. Conclusion

The transcription factor GATA6 was important in maintaining the differentiated state of epithelial cells in adult epithelium. Its absence was prone to epithelial cell transformation and polyploidy both were hallmarks prior

to carcinogenesis. Most of carcinomas had lost the expression of GATA6 prior to the hallmark of cancer and cell metastasis. The results of our study strongly put in evidence that the deficiency in GATA6 expression could be used as a biomarker to screen for women at risk of developing cervical cancer or already in primary phase of cervical cancer.

Acknowledgements

- All the hospital personnel in Cotonou (CNHU) and Parakou (CHD) who are taking care of women in gynecological services.

- Dr. Georges Offrin, who is the director of Hospital Mènontin in BENIN for his moral support throughout our investigations.

- Nurse Wannou Sylvia involved in sample collection for this study at Hospital Mènontin in BENIN.

- The laboratory of Pr. Xiang-Xi Xu from the University of Miami (USA)

- Master students (Lisette Degla, Ursula Houngue, Fiacre Akakpo and Aristide Gaba) and colleagues of the University Abomey-Calavi (BENIN) for their support as well as their input for the sample processing.

Statement of Competing Interests

This is an academic research work not funded by any organization therefore no competing interests was reported.

List of Abbreviations

BMCN: Biomarqueur Moléculaire en cancérologie et Nutrition (Biomarker in cancer and Nutrition)

CNHU: Centre National Hospitalier Universitaire (National University Hospital)

CHDU: Centre Hospitalier Départemental Universitaire (Departmental University Hospital)

CUS: cervical-uterine smears

HZ: Hôpital de Zone (Local Hospital)

LBBM: Laboratoire de Biochimie et Biologie Moléculaire (Laboratory of Biochemistry and Molecular Biology)

PBS: Phosphate buffered saline

RIPA: Radio immuno precipitation assay buffer

TBST: Tris buffered Saline,tween-20

TSA: trichostatin

References

- Zheng R., Blobel G.A. GATA Transcription Factors and Cancer. *Genes & cancer*, 1(12):1178-1188, December 2010.
- Smith E.R., Cai K.Q., Capo-Chichi C.D., Xu X.X. Aberrant epithelial differentiation in ovarian cancer. *Cancer treatment and research* 149:147-163, 2009.
- Bai H., Sakurai T., Godkin J.D., Imakawa K: expression and potential role of GATA factors in trophoblast development. *The Journal of reproduction and development*, 59(1):1-6,1 February 2013.
- Capo-Chichi C.D., Rula M.E., Smedberg J.L., Vanderveer L., Parmacek M.S., Morrisey E.E., Godwin A.K., Xu X.X: Perception of differentiation cues by GATA factors in primitive endoderm lineage determination of mouse embryonic stem cells. *Developmental biology* 286(2):574-586. October 2005.
- Haveri H., Westerholm-Ormio M., Lindfors K., Maki M., Savilahti E., Andersson L.C., Heikinheimo M. Transcription factors GATA-4 and GATA-6 in normal and neoplastic human gastrointestinal mucosa. *BMC gastroenterology*, 8(9):1-13, April 2008.
- Belaguli, N.S., Aftab, M., Rigi, M., Zhang, M., Albo, D., Berger, D.H. GATA6 promotes colon cancer cell invasion by regulating urokinase plasminogen activator gene expression. *Neoplasia (New York, NY)*, 12(11):856-865, November 2010.
- Molkentin, J.D. The zinc finger-containing transcription factors GATA-4, -5, and -6. Ubiquitously expressed regulators of tissue-specific gene expression. *The Journal of biological chemistry*, 275(50):38949-38952, December 2000.
- Capo-chichi, C.D., Roland, I.H., Vanderveer, L., Bao, R., Yamagata, T., Hirai, H., Cohen, C., Hamilton, T.C., Godwin, A.K., Xu, X.X. Anomalous expression of epithelial differentiation-determining GATA factors in ovarian tumorigenesis. *Cancer research*, 63(16):4967-4977, August 2003.
- Caslini, C., Capo-chichi C.D., Roland I.H., Nicolas E., Yeung A.T., Xu X.X. Histone modifications silence the GATA transcription factor genes in ovarian cancer. *Oncogene* 2006, 25(39):5446-5461, August 2006.
- Capo-chichi C.D., Cai K.Q., Testa J.R., Godwin A.K., Xu X.X. Loss of GATA6 leads to nuclear deformation and aneuploidy in ovarian cancer. *Molecular and cellular biology* 2009, 29(17):4766-4777, September 2009.
- Castellsagué X., Díaz M., de Sanjosé S., Muñoz N., Herrero R., Franceschi S., Peeling R.W., Ashley R., Smith J.S., Snijders P.J., Meijer C.J., Bosch F.X. Worldwide human papillomavirus etiology of cervical adenocarcinoma and its cofactors: implications for screening and prevention. *Journal of the National Cancer Institute*, 98(5):303-315, Mars 2006.
- Zhao F.H., Lewkowitz A.K., Chen F., Lin M.J., Hu S.Y., Zhang X., Pan Q.J., Ma J.F., Niyazi M., Li C.Q., Li S.M., Smith J.S., Belinson J.L., Qiao Y.L., Castle PE.: Pooled analysis of a self-sampling HPV DNA Test as a cervical cancer primary screening method. *Journal of the National Cancer Institute*, 104(3):178-188, February 2012.
- McEachin M.D., Xu X.X., Santoianni R.A., Lawson D., Cotsonis G., Cohen C. GATA-4 and GATA-6 synthèse in human ovarian surface epithelial carcinoma. *Appl Immunohistochem Mol Morphol*, 16(2):153-158. March 2008.
- Cai K.Q., Capo-Chichi C.D., Rula M.E., Yang D.H., Xu X.X. Dynamic GATA6 expression in primitive endoderm formation and maturation in early mouse embryogenesis. *Dev Dyn*, 237(10):2820-2829, October 2008.
- Yang D.H., Smith E.R., Cai K.Q., Xu X.X. C-Fos elimination compensates for disabled-2 requirement in mouse extraembryonic endoderm development. *DevDyn*, 238(3):514-523. March 2009.
- Lin L., Bass A.J., Lockwood W.W., Wang Z., Silvers A.L., Thomas DG, Chang AC, Lin J, Orringer MB, Li W, Glover TW, Giordano TJ, Lam WL, Meyerson M, Beer DG. Activation of GATA binding protein 6 (GATA6) sustains oncogenic lineage-survival in esophageal adenocarcinoma. *Proceedings of the National Academy of Sciences of the United States of America*, 109(11):4251-4256, March 2012.
- Cai K.Q., Caslini C., Capo-chichi C.D., Slater C., Smith E.R., Wu H., Klein-Szanto A.J., Godwin A.K., 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):6454, July 2009.
- Yang H., Lu M.M., Zhang L., Whitsett J.A., Morrisey E.E. GATA6 regulates differentiation of distal lung epithelium. *Development (Cambridge, England)*, 129(9):2233-2246. May 2002.
- Capo-Chichi C.D., Smedberg J.L., Rula M., Nicolas E., Yeung A.T., Adamo R.F., Frolov A., Godwin AK, Xu XX. Alteration of Differentiation Potentials by Modulating GATA Transcription Factors in Murine Embryonic Stem Cells. *Stem Cells Int.* 11; 2010:602068.