INK-4a Downregulated in *P53* and *P21* Mutation Induce BAT and VSMCs Proliferation: Obesity and Hypertension

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Abstract—

Background: *Epidemiology of obesity and hypertension are in high prevalence on low- and middle-neighborhood socioeconomic status (nSES) area. Later, the subjects become Diabetes and Chronic Kidney Diseases (CKDs) 1-5, and cancer due to p53, p21 and p16 mutation in AFB1 exposure population. Neglected p16/INK4a upregulated in early years which induce stunting, later induce proliferation of BAT (Uncoupling Protein UCPs132) or central Obesity, and vascular smooth muscle cells (VSMCs)/Hypertension.*

Aims: *p16 first upregulated in p21 and p53 mutation due to AFB1 exposure cause stunting, then when p16 downregulated in the older age induce cancer cells proliferation. This study recorded more the proliferation, reveal proliferation of BAT and VSMCs in healthy young age, in the downregulated p16 stage.*

Method: *Review article of Systematic Review and Meta-Analysis references of p16/INK-4a decreasing induced proliferation, also p16 Knock Out (KO) in p21/ p53 mutation population (AFB1 exposure).*

Result: *Mutation of p21/p53 in AFB1 exposure, induce p16/INK-4a downregulated in central Obese & VSMCs patients. Silencing/ downregulated p16/INK-4a induced proliferation, but still controversial with the upregulated in p53/p21 mutation (before).*

Discussion: *Proliferation of UCPs132 BAT cells, proliferation of SMCs, SGLT-2/GLP-1 therapy.*

Conclusion: *proliferation of BAT UCPs132 (central obesity) or VSMCs (hypertension) is due to downregulated p16 under p21/p53 mutation due to AFB1 exposure in low- and middle-nSES area population.*

Keywords— AFB1 exposure population, Hypertension, INK-4a, Obesity, p21/p53.

I. INTRODUCTION

There are so many controversies in Obesity and Diabetes, especially in nature or nurture,[1] geographical or eating habit,[2] also in p53-p21-p16 upregulated or downregulated in this high prevalence area. p16 is a protein that slows cell division, by slowing the progression of the cell cycle from the G1 phase to the S phase, thereby acting as a tumor suppressor. It is encoded by the P16 gene, the name of p16 is derived from its molecular weight 16 kD, and the similar name INK4 refers to its role in inhibiting CKD4 (cyclin-dependent kinase) involved in regulation of the cell cycle, act as tumor suppressor cells and stunting. Down regulated of p16/INK-4a (by p53 mutation) in UCPs 132,[3] VSMCs,[4] and later proliferation of cancer cells in AFB1 exposure population.[5] Uncoupling proteins are abundant in mitochondria of Brown Adipose Tissue (BAT) cells, function as thermogenesis metabolism, low of ATP synthesis, like the metabolism of hibernate bears.

The down regulation of p16 is controversial with upregulated p16Ink4, is as a suppressor protein that considered a tumor suppressor protein because both are high in p53 and p21 mutation in cancer patients and senescence.[5,6] The Paradoxical downregulated[7] of p16 mRNA with advancing age in BRAF-mutated polyps/adenomas indicates a senescence barrier in the serrated route to colon cancer,[8] unlike healthy cells. P16 upregulated as a compensate of the failure of p53 and p21 tumor suppressor cells.[6,7] The p16 pathway is a key regulator of the cell cycle, which controls the passage of cells from G, to S phase. P16 targets CDK4 and prevents Rb phosphorylation. Similar to p16 protein overexpression, is cause by viral E7 protein

(indirect marker of HPV-induced squamous cell carcinoma in head and neck (SCCHN).[9] The p16 gene is often mutated or epigenetically-silenced in SCCHN.[9] This made the survival rate low, and also the prognosis. P16 inhibits cancer cell growth by downregulating eEF1A2 through a direct interaction.[10] So, in p53 and p21 mutation in AFB1 exposure, p16 become the last defense, p16 +/+ or p16 -/- which induced senescence, also depends on Estrogen (year of age), to become Breast cancer after menopause years.[11,12] Guo et al, 2017, demonstrate that P16 may be associated with the Cigarette smoke extract (CSE)-induced proliferation of vascular smooth muscle cells (VSMCs),[13] suggesting that P16 serves a role in the development of CS-associated vascular diseases. Cell proliferation and cell cycle distribution were evaluate by flow cytometry, Western blotting for examine protein expression, and bisulfite genomic sequencing polymerase chain reaction was used to determine the hypermethylation of P16 promotor CpG island (repeat CGG).[14] Concentration- and time-dependent exposure induced a downregulation in P16 (all $P<0.05$).[13] Significant decrease in gene assay transcriptional activity reduced P16 protein expression in human aortic smooth muscle cells (HAOSMCs) (both $P<0.01$). Hypermethylation (silencing), mutation, or deletion leads to downregulation of the P16 gene.[14]

II. METHOD

Review article of p16 downregulated that induced proliferation, which supported UCPs132/ Brown Adipose Tissue (BAT) and VSMCs proliferation in stunting, obesity, and Diabetes Mellitus high prevalence area/ populations.[5] Using my Library, and academic search engine mainly ScienceDirect and EBSCOhost. Keywords of Bayesian network of p16 and downregulated were used, Systematic Review and Meta-Analysis references are preferable. P53-p21-p16 downregulated in AFB1 exposure food depends on keywords urine AFM1 in the population area of obesity and hypertension high prevalence. This epidemiology record is long before p16 downregulation is associated with AFB1 exposure in food, but it will be interesting that p16INK-4a downregulated induced UCPs132 cells and VSMCs proliferation in low- and middle-nSES population area.

III. RESULT

The UCPs132 BAT and VSMCs proliferation in AFB1 exposure population, should be supported by down-regulation of p16 gen or epigenetically. P53-p21 mutation due to AFB1 exposure, induced up-regulation of p16.[6] Paradoxical down-regulation of p16 mRNA with advancing age in AML[7] and GISTs[15] is similar to aging.[6] Table 1. describes p16 downregulation that induce proliferation, and the argumentation are supported as follows:

3.1 Epidemiology of p16 downregulation depends on estrogen/senescence:

The upregulation p16 induce stunting[16] but become downregulation after estro ER-/-/ senescence.[12] Childhood stunting is an important and intractable public health problem that cause about 20% of deaths among children age below 5 y in low- and middle-nSES population.[16] The evaluate interventions to limit exposure and reduce childhood stunting should be promoted. Senescence's after menopause happens because Estrogen promotes estrogen receptor negative BRCA1-deficient tumor initiation and progression in breast cancer.[12] Possible Down Regulation of the p16 gene promoter in individuals with carcinoma,[17] and give poor prognosis.[18] Hepato cellular carcinoma (HCC)[17] and breast cancer (BC) caused by AFB1 exposure has been broadly known[5]

3.2 Epidemiology meet Knock Out p16, induce proliferation:

Down-Regulated of the p16 Gene Promoter in Individuals with negative regulator of the cell cycle. In act of p16, especially promotor,[17] down-reg p16 expression predicts poor prognosis in patients with extrahepatic biliary tract carcinomas patients.[18] The immunohistochemically evaluated down-regulation of p16 in tumor specimen surgical has reported.[18] Over expressed p16, which normally inhibits cell proliferation, induces G1 cell cycle arrest in cervical cancer cells and precancerous lession.[19] The downregulation of p16 in SiHa and HeLa cells inhibited their proliferation, migration and invasion, also in cervical cancer.[19] p16 maybe a useful strategy in the diagnostic and treatment of cervical cancer.[19] P16 deficiency promotes tumor formation in various tissues.[20] Also induced leanness especially in old age, lower body weight as a protection against cerebellar senescence.[20]

3.3 p16 +/+ vs. +/- vs -/- and Estrogen Reseptor +/+ vs -/-:

p16 deficiency does not alter homeostasis in WAT, so induces leanness especially in old age.[20] Expression in estrogen receptor beta (ERB) also increase in deep cerebellar nuclei, implying cross talk between p16 and ERB. Protection against cerebellar senescence by promoting neuronal proliferation and homeostasis via ERB (in response to estrogen).[20]

p16 mRNA expression in T cells, a marker of cellular senescence, with BC are significant with the increase risk.[11] It is differed by age, race, family history of cancer, marital status, annual income, and smoking status,[11] which is represented to low- and middle-nSES.[5]

Wang[12] describe the distinctive features of senescent cancer cells and how these changes in proliferation and senolysis.[11,12] Also the deletion of cell cycle inhibitors p16 is required for development of Brca1-deficient basal-like mammary tumors in ER-positive vs. ER-negative, which estrogen stimulate proliferation and inisiating in both ER positive and ER-negative mammary tumor initiation and metastasis (independent of ER).[12]

3.4 Viral E7 protein to p16 down-regulation:

Viral E7 protein Loss of 9p, leads to p16 down-regulation and enables O-6-methylguanine-DNA methyltransferase (MGMT) promotes the anti-proliferative and pro-apoptotic when cervical cancer cells stimulated with 5-Aza-dC.[21] Methylation of p16 and MGMT was reversed. 5-Aza-dC inhibited E6 and E7expression and up-regulated p53, p21, and Rb expression.

HPV E6/E7 mRNA tests determine the oncogenic activity of the virus and represent a good clinical biomarker for predicting the risk of developing cervical cancer.[22] Also in Epstein Bar Virus (EBV) have shown increase cell proliferation.[23] Which is also paralleled liver Hepatitis B virus (HBV) DNA values.[24]

TABLE 1
DOWN-REGULATION P16 INDUCES PROLIFERATION

Reference/year	Type of p16 down-regulation	Cases	Effect	pathway
[7]de Jonge, 2009	P16 mRNA	AML	Aging: p16-p53→ proliferation	P16 up and then downregulation
[8]Kriegl, 2011	P16 expression	Polyp adenoma (senescence barrier)	Colon Cancer	
[15]Haller, 2008	P16INK4A and P16 mRNA	GISTS	Inhibits the CDK4 from phosphorylating RB	P16 located at 9p21
[6]Mijit, 2020	P53→p16	Aging	Upregulation p16	p53 mutation-AFB1exposure
[16]Smith, 2012	P16	Stunting	Upregulation-Estro-/-→downregulation	Food chain AFB1 exposure
[17]Shiraz, 2011	KO p16 (gene promoter)	HCC	Poor prognosis	P16 downregulation
[18]Ichikawa, 2002	P16 down-regulated	Tumor specimen surgical	Extra biliary tract cancer Prognosis	P16 downregulation
[5]Samsuria, 2018	P53 mutation	Ob-DM-HCC/BC	High prevalence	AFB1: P53-p21
[19]Zhang, 2014	P16 gene downregulation	Cervical Ca	Dx/Rx cervical ca	P16 downregulation
[26]Zhang 2015	P16 expression	Breast cancer	Effect of hypoxia	Fibroblast
[27]Zhang, 2021	LATS1: Large tumor suppressor kinase 1	VSCMs	Effect of CSE: Cigarette Some Extract	P16-G1 arrest
[20]Kim, 2019	P16 deficiency P16-Estrogen Receptor Beta	Various tissue Non WAT	Tumor formation Leanness in old age	P16 downregulated-Estrogen
[11]Shen, 2020	P16 mRNA	T cell senescence	Increase BC risk	Age, black, fam history of ca, nSES, smoking status
[12]Wang, 2022	P16 deletion	BC-after menopause	Senescence: change to proliferation	Estrogen Receptor independent
[21]Chen G-d, 2017	Methylation p16	Cervical cancer	Rx/ 5-Aza-dC inhibit E6/E7 exp	P53, p21, p16, Rb
[22]Sharma, 2022	HPV DNA and mRNA	Cervical cancer	proliferation	E6/E7
[23]Uehara, 2021	EBV and HPV double infection	Oral ca tissue samples	proliferation	Reduce P53 induction
[24]Pan, 2004	HBV	HPV mRNA and HBV	proliferation	Therapy
[25]Kumari, 2021	Upregulation p16	Through ROS, DNA damage, senescence	Aging cells process	P53/p21WAF1CIP1 and p16INK4A/pRB play a central role in regulating senescence.
[13]Guo, 2017	P16	CSE	VSMCs proliferation	
[14]Breuer, 2005	P16 promoter Bisulfite Genomic PCR	Hypermethylation	Concentration and time associated	CpG island (repeat CGG)

Activation of p16 (upregulated p16) through ROS, DNA damage, or senescence leads to the p16 in tissues, and is implicated in aging of cells,[25] BAT,[3] SMCs,[4] and Cancer cells.[18,19,20,21,30] This is about earlier upregulation of P16 gene-stunting then through senescence barrier, change to downregulation which induce proliferation. Conversely, p16 hypermethylation (silencing/KO), mutation, or deletion leads to downregulation of the gene and can lead to cancer through the dysregulation of cell cycle progression.

IV. DISCUSSION

CDKN (inhibitor)2A gene as the main tumor suppressor gene with p53 and p21, has another name P16/INK-4a and act as inhibiting CKD4 (cyclin-dependent kinase), with the help of hormone, antioxidant, antiviral. P16 induced by hypoxia are downregulated has been recorded. Table 2. Urine AFM1 (AFB1 exposure) in low- and middle-nSES home area describe the location of p16 downregulated high prevalence area. The mechanism are as followed:

4.1 Estrogen factors anti inflammation reaction:

Waist Circumference and Breast Cancer are associated with menstrual status[5] and Estrogen factors act as anti-inflammation protect to P16 down-regulation, inhibit proliferation.

4.2 HBV, EBV, HPV:

HBV. EBV, HPV infection without p16 down-regulation, suppressed tumor cells to proliferated.

Kras activation and p16 inactivation are required to develop pancreatic ductal adenocarcinoma (PDAC). Mutant Kras- and p16-regulated NOX4 activation overcomes metabolic checkpoints in development of PDAC.[28] Similar expression profile of KRAS and p16 reported in periampullary cancer.[30] In precancerous lession,[23]reveal the co-expression of low-risk HPV E6/E7 and EBV LMP-1 does not induce malignant transformation, but it allows accumulation of somatic mutations secondary to increase DNA damage and suppression of DNA DDR genes (DNA damage response and repair).[23]

4.3 Hypoxia induced proliferation:

Cigarette smoke extract (CSE) exposure, similar to hypoxis, down-regulated p16[13] and induce Vascular Smooth Muscle Cells (VSMCs) proliferation. The role of P16 in CSE-induced VSMCs proliferation and the underlying mechanism. also has been reported in the CKD1 in low- and middle-nSES AFB1 exposure population/ p53 mutation.[3,4,5]

4.4 Time-dependent exposure:

Time-dependent exposure induced p16 downregulation-proliferation of precancer cells, BAT cells mitochondrial rich UCPs132 and SMCs proliferation supported.[13] Not only time-exposure, but the level concentration of exposure are also recorded.[13]

4.5 Reduce p16 expression in HAOSMCs:

Activation of p16 (upregulated p16) through ROS, DNA damage, or senescence leads to the buildup of p16 in tissues and is implicated in aging of cells.[25] P53/p21WAF1CIP1 and p16INK4A/pRB play a central role in regulating senescence.[25] Boys and Girls in Sierra Leone, West Africa, frequently and constantly exposed by AFB1.[32] Birth weight of infant is discussed, and the cord blood aflatoxin found in 58% pregnant woman.[32] After the senescence reveal by stunting due to upregulated p16, reduce genetic or epigenic p16 expression induced proliferation in specific tissue e.g. human aortic smooth muscle cells (HAOSMCs),[13] UCP132 (BAT),[3] and fibroblast.[26] The loss of p16 expression due to p16 promotor hypermethylation occurs late in carcinogenic process at the level of severe dysplasia.[14]

4.6 Fibroblast migration and invasion:

Fibroblast migration and invasion in 83% breast CAFs compared primary cells and also in breast cancer tissues in hypoxia is reported.[26]

Up and downregulation of p16 expression in BRAF-mutated polyps/ adenomas indicates a senescence barrier in the Colorectal carcinoma (CRC) process.[8] P16 hypermethylation, mutation, or deletion leads to downregulation of the gene and can lead to cancer through the dysregulation of cell cycle progression. The liberates E2F1 (Transcription Factor) from its bound state in the cytoplasm and allows it to enter the nucleus. Once in the nucleus, E2F1 promotes the transcription of target genes that are essential for transition from G1 to S phase.[15] This pathway connects the processes of tumor oncogenesis and senescence, fixing them on opposite ends of a spectrum. All Obesity and Hypertension high prevalence populations, are in the high prevalence of

stunting with AFB1 exposure-p53 mutation.[5 and 16] Urine AFM1 describe AFB1 exposure in low- and middle-nSES home area had high Obesity (BAT proliferation) and Hypertension (SMCs proliferation) prevalence.

TABLE 2
IDENTIFIED LITERATURES ON URINE AFM1 (AFB1 EXPOSURE) IN LOW- AND MIDDLE-nSES HOME AREA

Study, year	Area of interest	Adjustment of interest	Comparative urine AFM1	Comorbidities influence	Influence of LC-MS/MS
[33]Ali, 2016	Hot and Humid climate	Bangladesh	Rural vs. Urban	Adult, children	Urine AFM1 as biomarker of AFB1 exposure
[34]Gerding, 2015	Germany	23 mycotoxins	Bangladesh Germany Haiti	Prevention of harmful health	Bangladesh and Haiti only, not Germany
[35]Jager 2016	UPLC-MS/MS	Brazil	HPLC with fluorescence detection	Prevention of harmful health	Confirmed urine AFM1 very sensitive for AFB1 exp
[36]Ezekiel, 2014	Rural North Nigeria	LC-MS/MS multi biomarker	Children Adolescents Adults	Major Public Health Challenge	Call for urgent intervention
[37]Warth, 2014	Bangkok	Max AFB1	LC-MS/MS urine AFM1	4 urine biomarkers	First in SEA
[38]Mitchell, 2013	AFB1 is a persistent public health issue in Ghana	Urine AFM1	UPSN vs. placebo	intervention	UPSN reduced AFM1 biomarker
[39]Solfrizo, 2011	1st time reported	High pressure LC-MS/MS	Urinary biomarkers	Aflatoxin exposure could be measured	Urine AFM1 can be used
[40]Solfrizo, 2014	AFB1 exp	Urine AFM1	UPLC MS/MS in pg/mL	Southern Italy ZEA 100%, AFM1 6%	Urine AFM1 detected
[41]Ahn, 2010	Quantitative	Urine AFM1 in pg/ml	LC-MS/MS	Using immunoaffinity collumn	Korean population 1/12 detected
[42]De Cassia, 2009	Brazillian population	Urine AFM1	UPLC low pressure with fluorescence detection	Food contamination	AFB1 exposure
[31]Jonsyn, 2001	Seasonal observational	Urine AFM1 from children in Sierra Leone	Dry and Rainy season	Boys and girls	Frequently and constantly exposed
[43]Shephard, 2013	1st void morning urine	Urinary multi-mycotoxin	LC-MS/MS	Esophageal cancer region	Maize-based evening meal
[44]Warth, 2012	Quantitative in sub-ppb	AFM1 in Cameroon	LC/ESI-MS/MS	In human urine	Key metabolite
[45]Chen, 2017	Aptamer AFB1	Fluorescence enhancement	LOD 1.6 ng/ml	DNA strand containing a quencher moiety	+
[46]Jonsyn, 1999	Aflatoxin	HPLC specimen of infants	High contamination rate	AFB1 G2, OTA, OTB	Urine sample were 100% contaminated
[47]Jonsyn, 2007	Aflatoxin	Urine sample of school children	High concentration level	57% serum samples + aflatoxin	Low compared to urine
[32]Jonsyn, 1995	Cord blood sample from pregnant	OTA	OTA in 25% Aflatoxin in 58%	No urine AFM1	Birth weight of infant

V. LIMITATION:

Many study didn't specify proliferation after stunting (aging) but report proliferation due to $p16^{INK4a}$downregulation due loss of 9p chromosome of p21.[15] p16(INK4a) upregulated up to p16 downregulation by epimutation/ hypermethylation should be known by the researchers.[14]

This study also didn't specify age-dependent p16 epimutation, but proposes as the therapeutic target for colorectal cancer.[48] And KRAS, p53 are on mutation sequence before DCC gene,[49] similar to what KRAS activation and p16 downregulation in other precancer.[28,30] Mutant Kras- and p16-regulated NOX4 activation overcomes metabolic checkpoints in development of Pancreatic ductal adenocarcinoma.

This study also didn't specify that p16 downregulation depends on senescence, while Wang reported exploiting senescence for the treatment of cancer,[50] where 5-Aza-dC is reveal for the treatment of cancer based on hypermethylation of p16 promotor.[21] Stunting/ senescence due to p53 mutation.[16] 5-Aza-dC is also reported to other broad range detrimental health cause by hypermethylation other than cancer.[51]

This study also didn't characterize Sodium-Glucose Cotransporter 2 (SGLT2) Inhibitors as therapy of afferent contraction due to VSMCs proliferation on afferent glomerulus.[52, 53, 54] The area of AFB1 exposure could be found in low- and middle nSES (income) in Table 2,[56] especially in children and infant and moreover since pregnant women.[57,58,59,60,61] which have associated to growth impairment.[60,62,63,64] In children, adolescence and adults, is associated with major public health challenge, incl. HIV.[36] It is emphasize that AFB1 especially in utero, associated to DNA methylation in white blood cells of infants in the Gambia.[64]

Mild, moderate, severe stunting & underweight on Systematic Analysis in 141 developing countries,[61,65,66,67,] is completed by Sousa reported stunting and overweight/obese high prevalence in Brazilian children (Systematic Review and Meta-Analysis),[68] and Romero report Urine AFM1 in Brazilian population associated with food consumptions parallel with the highest Diabetes Melitus (IDF), CVD (WHO).[42] Stern 2001 reported HBV and AFB1 exposure due to p53-codon 249 mutation in China with Meta-Analysis.[69] It is supported by chronic hepatomegali,[70] aflatoxin cause stunting in Benin,[71] Aflatoxin exposure in young children Benin and Togo, West Africa,[71] complete this finding of AFB1 exposure induce the proliferation by downregulation of p16INK4a after stunting.[5,16] p53-p21-p16 axis,[73] p16 deletion,[50] p16 methylatioon,[21] which VSMCs function in atherosclerosis-DNA methylation.[55]

VI. CONCLUSION

Proliferation of BAT UCP132 (obesity) or SMCs (hypertension) is due to downregulated p16 under p21/p53 mutation due to AFB1 exposure in low- and middle nSES area population with high childhood stunting (cell senescence) prevalence.

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CONFLICT OF INTEREST

Nothing

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