ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of Development Research
Vol. 11, Issue, 09, pp. 50622-50625, September, 2021
https://doi.org/10.37118/ijdr.23000.09.2021

RESEARCH ARTICLE OPEN ACCESS

AURORA KINASES EXPRESSION AND POSSIBLE CANCER RISK INDICATOR IN INDIVIDUALS WITH DIABETES MELITTUS TYPE 2

[1]Mariana Neves Tavares, [1]Carla Silva Siqueira Miranda, [1]Wagner Gouvêa dos Santos, [1]Marianne Lucena da Silva, [2]Katiane da Costa Cunha, [3]Fermino Sanches Lizarte Neto, [1]Aline Monezi Montel and [1]Fábio Morato de Oliveira

[1]Federal University of Jataí, Cidade Universitária, BR 364, km 195, nº 3800; [2]State University of Pará, Belém, Pará, Brazil; [3]Faculty of Medicine of Ribeirão Preto, University of São Paulo, Brazil

ARTICLE INFO

Article History:
Received 10th August, 2021
Received in revised form
17th August, 2021
Accepted 03rd September, 2021
Published online 30th September, 2021

Key Words:
Aurora Kinase Genes, Diabetes, Gene Expression, Genomic Instability, *TP53* gene.

***Corresponding author:**
Dr. Fábio Morato de Oliveira

ABSTRACT

We aimed to verify the genomic instability in type 2 diabetes mellitus (DM2) by comparing the levels of *AURKA* and *AURKB* expression, commonly hyperexpressed in many tumors, in addition to the *TP53* deletion status. Peripheral blood samples from 76 individuals, being 36 samples from individuals with DM2 and 40 samples from health donors were used. Significant differences were observed (*AURKA*: 2.013 ± 0.322 *versus* 1.126 ± 0.217, $p < 0.0001$, in DM2 *versus* health donors) and (*AURKB*: 1.600 ± 0.113 *versus* 1.133 ± 0.121, $p < 0.0001$, in DM2 *versus* health donors). All results were confirmed by FISH. Regarding the *TP53* status, only 19 individuals (52,7%), from DM2 group, showed TP53 deletion. Although the over expression of aurora kinase genes presents an oncogenic potential, it is not possible to conclude that an increase in the expression of these genes would necessarily lead to the development of a neoplasm in these patients. However, our results suggest that some individuals in the DM2 may have higher risk for genomic instability.

Citation: ***Mariana Neves Tavares, Dra. Carla Silva Siqueira Miranda, Dr. Wagner Gouvêa dos Santos, Dra. Marianne Lucena da Silva et al.*** "Aurora kinases expression and possible cancer risk indicator in individuals with diabetes melittus type 2*", International Journal of Development Research*, 11, (09), 50622-50625.

INTRODUCTION

Besides being related to vascular complications, the poor glycemic control in type 2 diabetes mellitus (DM2) has also been associated with increased cancer risk. The strongest associations are with breast and endometrial cancer in women and colorectal and intrahepatic (liver) cholangiocarcinoma in both sexes (Larsson SC, 2007; IDF, 2019). However, the relationship of DM2 and cancer remains unclear. In DM2 the main factors suspected to interfere with carcinogenesis are hyperglycemia and hyperinsulinemia thought to cause cellular damage from glucotoxicity, lipotoxicity and oxidative stress (Cignarelli *et al.,* 2018). It has been demonstratedthat patients with DM2, compared to the control group, show a higher value of micronuclei, nucleoplasmic bridges and nuclear buds, which are not necessarily markers of cancer, but are structures that correlate to the levels of genotoxicity and DNA damage, which can result in mutagenic effects (Salimi *et al.*, 2016).

The progression of cell division is controlled by a complex molecular machinery of proteins present during checkpoints to correct possible errors. The *TP53* tumor suppressor protein is well established as a barrier to neoplastic transformation and tumor progression and missense mutations in the TP53 gene are commonly found in human cancers (Mantovani, Collavin & Del Sal, 2019). On the other hand, aurora kinases are serine/threonine kinase enzymes that regulate chromosomal alignment and segregation during mitosis and meiosis (Carmena *et al.,* 2015). The expressions of *AURKA* and *AURKB* genes are usually detectable in somatic cells with a high rate of proliferation, as in hematopoietic cells, while Aurora Kinase C is expressed in germ cells (Carmena *et al.,* 2015). Additionally, overexpression or amplification of aurora kinases have been demonstrated in several types of cancer, including different forms of leukemias and solid tumors (Yang *et al.,* 2010; Zekri *et al.,* 2012; Goldenson and Crispino, 2015). Therapeutic inhibition of aurora kinase has been showing increasingly promising as strategy for the treatment of cancer, which demonstrate its great importance in the process of cell division (Goldenson and Crispino, 2015). Aurora

kinases inhibitors can effectively repress the progression and growth of cancers as demonstrated both *in vitro* as *in vivo* (Tang *et al.,* 2017). Herein we investigated, for first time, the levels of *AURKA* and *AURKB* expression in individuals with DM2, in addition to TP53 status, compared to individuals without DM2, as an approach to determine the potential risk for genomic instability in both groups.

MATERIAL AND METHODS

Samples: Peripheral blood samples were collected from 76 individuals, divided into two groups, being 36 samples from individuals with DM2 and 40 samples from individuals without DM2 (health donors). All samples were provided by the Ambulatory of Stomatological Diseases of the Southwest of Goias (ADESGO), through a partnership between the Federal University of Jataí (UFJ), and the Municipal Health Secretariat of Jataí – GO, Brazil. The samples were collected between August and December of 2018. The ambulatory aims to enable the detection of lesions relevant to the early diagnosis of stomatological diseases, and contribute to the reduction of mortality and morbidity from oral cancer. The study was approved by the Ethics Committee of the Federal University of Jataí, Goiás, Brazil (69791717.0.0000.5083) andall the participants were invited to sign a copy of the informed consent form, also signed by the main investigator, containing general information about the study.

Gene expression profile analysis: Genomic RNA was isolated from peripheral blood cells using TRIzol reagent (Invitrogen) according to the manufacturer's recommendations. Complementary DNA (cDNA) was synthesized from ~1µg of total RNA using a High-Capacity cDNA reverse transcription Kit (Thermo Fisher Scientific, Waltham - Massachusetts, EUA), following the manufacturer's instructions. For analysis of aurora kinase genes, primes and probe developed by Assay on Demand were used (*AURKA*: Hs00269212_m1 and *AURKB*: Hs00177782_m1; Thermo Fisher Scientific). The *AURKA* and *AURKB* genes and *GAPDH* mRNA, used as endogenous internal control for each sample, were analyzed in duplicate on the same MicroAmp optical 96-well plates using a 7500 Real-Time PCR System (Thermo Fisher Scientific, Waltham - Massachusetts, EUA). Real-time quantitative polymerase chain reaction (RT-qPCR) assays were performed in a final reaction volume of 20µl. The comparative cycle threshold (Ct) method was used to determine the relative expression level of *AURKA* and *AURKB* genes. The comparative analysis between DM2 and healthy donors' samples was calculated by quantification of the *AURKA* and *AURKB* gene expression relative to the expression of *GAPDH* housekeeping gene The *AURKA* and *AURKB* gene expression in DM2 sampleswere calculated as relative quantification to normal controls ($\Delta\Delta Ct = \Delta Ct_{\text{patient}} - \Delta Ct_{\text{healthy donors+}}$) and expressed as $2^{-\Delta\Delta Ct}$. Comparisons between different groups were made using the Student *t* test and the two-sided exact Fisher test (dichotomous variables) (GraphPad Prism 8). *P values* < 0.05 were considered significant.

Fluorescence in situ hybridization (FISH): Fixed cells were prepared and hybridized according to the manufacturer's instructions. The hybridization spots were evaluated using a Nikon E-200 microscope (NIKON, EUA) equipped with a set of filters and a software for capture and documentation analysis. Acommercial set of probes (AURKA: ON AURKA (20q13)/20q11 and AURKB: AURKB (17/p13)/SE17; Kreatech Diagnostics, Amsterdam, The Netherlands) were used to confirm the elevated copy number of *AURKA* and *AURKB* genes. The probes were designed as a dual-color assay to detect amplification at 20q13 and 17p13, respectively. Amplification involving these genes regions will show multiple red signals, while the controls (MPARE1 for AURKA and SE17 for AURKB), both located in the centromeric region of their chromosomes, will provide 2 green signals. The criteria used for *AURKA* and *AURKB* genes amplifications were based on the number of spots presented during analysis. For *TP53* analysis we used the TP53 (17p13) specific FISH probe, which is optimized to detect deletion of the TP53 gene region at 17p13. Deletions involving the *TP53* gene region will show one green signal and two red signals at the chromosome 17 centromere control region (1R2G). For each DM2 sample it was analyzed 200 interphase nuclei to measure deletions of the *TP53* gene.

RESULTS

Seventy-six blood samples, divided into two groups, being 36 samples from individuals with DM2 (22 males and 14 females; median age 56,5 years, range 42–65,2 years) and 40 from healthy individuals were used for gene expression analysis (Table 1). We compared the expression profile of *AURKA* and *AURKB* in peripheral blood cells from DM2 individuals and healthy donors. Statistically significant differences were observed in the two groups (*AURKA* [mean value of $2^{-\Delta\Delta Ct}$ ± SD]: 2.013 ± 0.322*versus*1.126 ± 0.217, $p<$ 0.0001, in DM2 group *versus* health donors) (Figure 1A).

Table 1. Biochemical profile, smoking habit, age, sex, and sample size of the studied groups

	DM2 individuals	*Control group*
Sample size	36	40
Male	22	29
Female	14	11
Age mean ± SD	56,5 ± 7,9	49,3 ± 5,8
Smokers	-	-
Fasting glucose in mg/dl - median (95% CI)	252,34 (157,4 - 294,2)	-

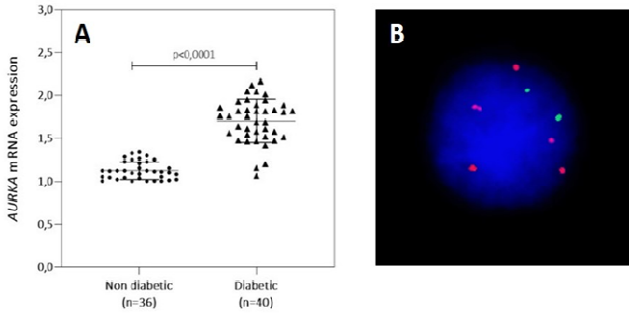

Figure 1. qPCR analysis of *AURKA* mRNA expression and interphase FISH analysis in DM2 group, compared with health control. (A) The graph represents the mean SD of three independent experiments. The p values are indicated in the graphs; p<0.0001; ANOVA test and Bonferroni post-test were performed. (B) Interphase FISH analysis of *AURKA* gene demonstrating elevated DNA copy number in DM2 group (spots in red indicate *AURKA* amplification, green spots correspond to control centromeric region)

The elevated expression of *AURKA* in DM2 group was confirmed by FISH using a commercial probe for *AURKA*gene (Figure 1B).

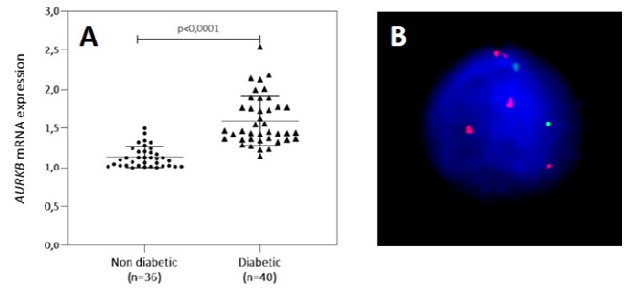

Figure 2. qPCR analysis of *AURKB* mRNA expression and interphase FISH analysis in DM2 group, compared with health control. (A) The graph represents the mean SD of three independent experiments. The p values are indicated in the graphs; p<0.0001; ANOVA test and Bonferroni post-test were performed. (B) Interphase FISH analysis of *AURKB* gene demonstrating elevated DNA copy number in DM2 group (spots in red indicate *AURKB* amplification, green spots correspond to control centromeric region)

By using the same stratification criteria for the participants in the study, we also found significant differences in gene expression for *AURKB*. However, the difference in the levels of expression were more discrete than those observed for AURKA(*AURKB* [mean value of $2^{-\Delta\Delta Ct} \pm$ SD]: 1.600 ± 0.113*versus*1.133 ± 0.121, $p < 0.0001$, in DM2 group *versus* health donors) (Figure 2A). The results obtained were confirmed by FISH, by using a commercial probe for *AURKB* gene (Figure 2B). For the TP53 FISH analysis we found that 19 DM2 individuals (52,7%) presented deletion. However, the frequency of cells displaying TP53 deletion was less than 10% (Figure 3).

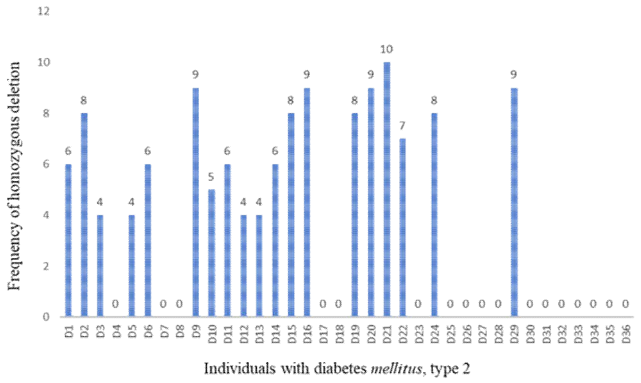

Figure 3. FISH analysis for TP53 deletion. The graphic shows the frequency of homozygous deletion involving the TP53 gene in DM2 group. For each sample were analyzed 200 interphase nuclei

DISCUSSION

Several studies have shown an increased cancer risk in diabetes patients compared to respective controls (Cignarelli et al., 2018; Larsson et al., 2007). In fact, patients with type 2 diabetes, have a 20–40% increased risk for colorectal cancer in comparison with non-diabetic persons (Wojciechowska *et al.,* 2016). A prospective study selected patients with the five main chronic diseases, such as diabetes and lung disease, together with some disease markers (blood pressure, total cholesterol, and heart rate) to assess their possible correlations with increased cancer risk. The results confirmed that these chronic diseases, risk factors commonly neglected in cancer prevention, really have a great impact, since they contributed to more than a fifth of cancer incidences and more than a third of cancer deaths (Tu *et al.,* 2018). This study aimed to identify a possible association between the level of expression and mutation profile of some genes involved in cell cycle regulation in diabetes melittus type 2 individualsand the increased risk for cancer. Genomic instability is known to be part of the oncogenesis and we hypothesized that differential expression of *AURKA* and *AURKB* genes could be shown in DM2 patients.

Considering that elevated expression of *AURKA* and *AURKB* is frequently observed in a wide variety of cancers, this evidence strongly indicate that high expression of these genes is somehow important for the development of cancer associated phenotypes (Carmena *et al.,* 2015). However, it is important to note that t none of the patients recruited for this study had been diagnosed with cancer. Nevertheless, we found significant differences in quantitative expression of *AURKA* and *AURKB* genes in DM2 patients, when compared with health individuals.The meaning of these differences deserves further studies since DM2 patients present a metabolic syndrome and dysregulated cell function. Oxidative stress due increased metabolism and potential DNA damage promoted by increased cell division in a *TP*53 mutant background may lead to genomic instability and the risk of developing secondary diseases, such as cancer. *AURKA* has been demonstrated to act as an oncogene when over expressed in mammalian cells *in vitro*, there is also suggestive evidence of high *AURKB* expression being oncogenic *in vivo* (Carmena *et al.,* 2015). It is important to keep in mind that these two proteins are important regulators of mitosis, thus cells over expressing *AURKA* and *AURKB* frequently manifest aneuploidies. Although it was not possible to perform classical cytogenetic analysis in all DM2 patients, for twelve of them, with abnormal levels of *AURKA* and *AURKB*, we found a normal karyotype (*data not shown*). Overexpression of *AURKA* induces increased cell cycle progression, development of genomic instability and aneuploidy. The dysregulation of the expression of this gene is gaining increased clinical relevance, as it was an alteration identified in several common cancers and has also been used as a possible marker of poor prognosis. There are several reports correlating the amplification and overexpression of aurora kinase geneswith progression of carcinogenesis (Mobley *et al.,* 2017). The expression of *AURKB* also has been reported as clinically significantsimilarly to *AURKA* and is hyperactive in most aneuploid tumors. In hepatocellular tumors, the overexpression of *AURKB* served as a marker of tumor invasiveness and, also, of a worse prognosis. Likewise, the expression of aurora kinase B was higher in non-small cell carcinoma compared to samples with normal epithelium (Willems *et al.,* 2018).

It is known that more than half of human cancers present abnormalities in *TP53* gene or in genes whose activity control *TP53* (*MDM2* or *CDKN2A*). The *TP53* gene acts as a transcription factor to transactivate gene expression following genotoxic or oncogenic stress. Under mild stress, activated *TP53* induces expression of growth-arrest genes, such as *CDKN1A/p21*, to facilitate DNA damage repair (Minamino *et al.,* 2009). Important evidence connecting the *TP53* gene, and the development of type 2 diabetes was demonstrated when the inhibition of TP53 activity, either by siRNA knock-down in cells, or by *TP53* gene knock-out in mice, alleviated senescence and caused decreased inflammatory cytokine expression in the adipose tissue of mice, ultimately preventing them from developing insulin resistance (Tavana *et al.,* 2010). Later, in a study focusing on the link between non-homologous end-joining (NHEJ) DNA repair mechanisms and *TP53* it was discovered another unexpected connection between *TP53* and diabetes (Tavana *et al.,* 2010). The knockout of Lig4 (DNA Ligase 4) in mice resulted in NHEJ deficiency and embryonic lethality; not surprisingly, this embryonic lethality was rescued by *TP53* deficiency. However, the result was severe diabetes and early fatality in these mice; these were attributed to senescence of the pancreatic beta cells in mice model. Our results demonstrated a low frequency of *TP53* deletion in DM2 group (less than 10%), in only 19 individuals. However, it is important to note that this gene is known to be susceptible to a variety of different mutations, majority of them was not covered in our assay. The chronic hyperglycemic and hyperlipidemic features present in DM2 patients lead to oxidative stress, which is also involved in the progression of the disease and contributes to oxidation of DNA. Cells exposed to various concentrations of different DNA damaging agents accumulate postmitotic damage and display signs of senescence. Latter phenomenon is named as stress induced premature senescence (SIPS). In this scenario, unresolved and accumulated reactive oxygen species can induce an inflammatory response and the expression levels of many genes are changed during SIPS.

Thus, it is believed that, in response to excessive production of free radicals, cellular and molecular mechanisms promote activation of cell cycle genes, that may also involve aurora kinase genes, not only promoting DNA damage, but also elevating the degree of genomic instability. In opposite the accumulating genomic instability, the resulting DNA damage activates DNA damage sensors like tumor suppressor *TP53* and cell cycle checkpoint kinases, to repair damaged DNA. Notably, *TP53* is known to promote genomic stability via ATM/ATR pathway and cell cycle arrest to allow the repair of DNA damage or to cause cell death (Karaman *et al.,* 2015). In healthy cells the activity of auroras kinases is essential for regulating mitosis and for a correct control of the cell cycle. Although, its massive overexpression has an oncogenic potential, and, in addition, it is associated with phenotypes of greater malignancy and worse prognosis in tumors.According to our results it is not possible to conclude that an increase in the expression of aurora kinase genes would necessarily lead to the incidence of a neoplasm in DM2 patients.However, we must have in mind that the altered expression

of *AURKA/B* genes shown here suggests a warning for the possibility of greater genomic instability in the population with diabetes.

Conflict of Interest: The authors declare that they have no conflict of interest.

REFERENCES

Carmena M, Earnshaw WC, Glover DM. 2015. The Dawn of Aurora Kinase Research: From Fly Genetics to the Clinic. *Front Cell Dev Biol.,* 3:73.DOI: 10.3389/fcell.2015.00073

Cignarelli A, Genchi VA, Caruso I, Natalicchio A, Perrini S, Laviola L, Giorgino F. 2018. Diabetes and cancer: Pathophysiologicalfundamentals of a 'dangerousaffair'. Diabetes Res Clin Pract. 143:378-388. DOI:10.1016/j.diabres.2018.04.002

Goldenson B, Crispino JD. 2015. The aurora kinases in cell cycle and leukemia. Oncogene. 34(5):537-45. DOI: 10.1038/onc.2014.14

International Diabetes Federation. IDF Atlas. 9th ed. Brussels, Belgium: International Diabetes Federation (IDF); 2019.

Karaman A, Aydın H, Geçkinli B, Çetinkaya A, Karaman S. DNA damage is increased in lymphocytes of patients with metabolic syndrome. Mutat Res Genet Toxicol Environ Mutagen. 2015; 782:30-5. DOI: 10.1016/j.mrgentox.2015.03.009

Larsson SC, Mantzoros CS, Wolk A. Diabetes mellitus and risk of breast cancer: a meta-analysis. Int J Cancer. 2007 Aug 15;121(4):856-62. DOI: 10.1002/ijc.22717.

Mantovani, F., Collavin, L. & Del Sal, G. Mutant p53 as a guardian of the cancer cell. Cell Death Differ 26, 199–212 (2019). https://doi.org/10.1038/s41418-018-0246-9

Minamino T, Orimo M, Shimizu I, Kunieda T, Yokoyama M, Ito T, Nojima A, Nabetani A, Oike Y, Matsubara H, et al. A crucial role for adipose tissue p53 in the regulation of insulin resistance. Nat Med. 2009; 15:1082–1087. DOI: 10.1038/nm.2014

Mobley A, Zhang S, Bondaruk J, Wang Y, Majewski T, Caraway NP, Huang L, Shoshan E, Velazquez-Torres G, Nitti G, Lee S, Lee JG, Fuentes-Mattei E, Willis D, Zhang L, Guo CC, Yao H, Baggerly K, Lotan Y, Lerner SP, Dinney C, McConkey D, Bar-Eli M, Czerniak B. Aurora Kinase A is a Biomarker for Bladder Cancer Detection and Contributes to its Aggressive Behavior. Sci Rep. 2017; 7:40714. DOI: 10.1038/srep40714

Salimi M, Broumand B, Mozdarani H. Association of elevated frequency of micronuclei in peripheral blood lymphocytes of type 2 diabetes patients with nephropathy complications. Mutagenesis. 2016; 31(6):627-633. DOI: 10.1093/mutage/gew029

Tang A, Gao K, Chu L, Zhang R, Yang J, Zheng J. Aurora kinases: novel therapy targets in cancers. Oncotarget. 2017 Apr 4;8(14):23937-23954. DOI: 10.18632/oncotarget.14893.

Tavana O, Puebla-Osorio N, Sang M, Zhu C. Absence of p53-dependent apoptosis combined with nonhomologous end-joining deficiency leads to a severe diabetic phenotype in mice. Diabetes. 2010; 59:135–142. DOI: 10.2337/db09-0792

Tu H, Wen CP, Tsai SP, Chow WH, Wen C, Ye Y, Zhao H, Tsai MK, Huang M, Dinney CP, Tsao CK, Wu X. Cancer risk associated with chronic diseases and disease markers: prospective cohort study. BMJ. 2018; 360:k134. DOI: 10.1136/bmj.k134

Willems E, Dedobbeleer M, Digregorio M, Lombard A, Lumapat PN, Rogister B. The functional diversity of Aurora kinases: a comprehensive review. Cell Div. 2018; 13:7. DOI: 10.1186/s13008-018-0040-6

Wojciechowska J, Krajewski W, Bolanowski M, Kręcicki T, Zatoński T. 2016. Diabetes and Cancer: a Review of Current Knowledge. *Exp Clin Endocrinol Diabetes.* 124(5):263-75. DOI: 10.1055/s-0042-100910

Yang G, Chang B, Yang F, Guo X, Cai KQ, Xiao XS, Wang H, Sen S, Hung MC, Mills GB, Chang S, Multani AS, Mercado-Uribe I, Liu J. 2010. Aurora kinase A promotes ovarian tumorigenesis through dysregulation of the cell cycle and suppression of BRCA2. *Clin Cancer Res.,* 16:3171–81.DOI: 10.1158/1078-0432.CCR-09-3171.

Zekri A, Lesan V, Ghaffari SH, Tabrizi MH, Modarressi MH. 2012. Gene amplification and overexpression of Aurora-C in breast and prostate cancer cell lines. *Oncol Res.,* 20:241–50.

****