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Research Article

Oncogenic Characteristics, Functional Partners and Protein Structure Variations in Isolated MDM2 Gene among Liver Cancer Patients in UCTH, Calabar, Nigeria

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Abstract

This research investigated the oncogenic properties and protein structure variations of mutations in the mouse double minutes 2 (MDM2) gene implicated in liver cancer patients in the University of Calabar Teaching Hospital, Calabar. Forty five liver cancer patients and fifty controls were recruited for the study. Blood samples were collected for molecular analysis. DNA was isolated from the blood samples of all the subjects and sequenced for the presence of MDM2 gene mutation. The chromatogram was decoded into nucleotide sequences using Chromas Pro and later translated to protein sequences. Bioinformatics tools were used to unveil the oncogenic characteristics, functional partners of MDM2 gene/protein interactions and the MDM2 protein structure variations. The result revealed significant differences in oncogenic characteristics as molecular weights, theoretical isoelectric points, total number of atoms, size of gene, G-C content, extinction coefficient, estimated half- life, instability index, aliphatic index, total positively and negatively charged amino acids and hydropathicity of the MDM2 gene for both patient and control group isolates. The results of MDM2 gene oncology /protein interactions revealed strong relationship and interactions with functional partners of major genes and proteins such as MDM4, TP53, TP57, RLP11, EP300, UPST and CDKN2A proteins. Results of secondary protein structures for the MDM2 gene isolates showed variations in the percentage distribution of alpha helices, beta sheets, extended strands and random coil. The tertiary protein structure further revealed the double mouse shape of the gene which accounts for the name mouse double minutes 2 gene. Knowledge of the oncogenic characteristics of the mutated and non-mutated MDM2 gene and the variations that exist in the protein structure characteristics can play a major significant role in the design and development of therapy for liver cancer disease and further help to unmasked the genetic factors and interactions that trigger the loss of functions and deviant expressivity of the mutated MDM2 gene among patients. Hence, the findings of this study serve as baseline information for further studies on the genetic etiology of the disease.

Keywords: MDM2 Gene Oncology; Alpha Helices; Tertiary Structure; Molecular Characteristics; Biological Process

Introduction

Advances in genomic studies have increased the knowledge of Hepatocellular carcinoma (HCC) molecular pathogenesis in different populations [1-3]. Several studies have analyzed distribution of single nucleotide polymorphism in genes encoding for cell cycle regulatory proteins and susceptibility to malignant diseases [4,5]. There is rapid increase in knowledge and understanding of cancer diseases and their respective genetic underpinnings. As a result, scientists have mapped many genes and their variants that can influence the molecular pathogenesis

of hepatocellular carcinoma [6,7]. Collaborative molecular genetics studies conducted by oncologists and human geneticists during the last several years have thrown some light on the molecular pathogenesis, clinical and genetic presentations of hepatocellular carcinoma [8-12]. Studies have indicated the association of MDM2 gene mutation in the molecular pathogenesis of hepatocellular carcinoma [13-15] and other cancers [16-18]. Hepatocellular carcinoma (HCC) is a complication of liver cirrhosis caused by hepatitis A, B, C viral infection, fungal infection and genetic factors [19-22].

Mutations within the genome trigger the progression of liver cancer and some mutations are commonly observed across multiple cancer patients while others are less frequent, representing heterogeneity within the mutational landscape [23-25]. Liver cancers and other forms of cancers in patients show a high amount of somatic mutations of approximately 50 per tumor [26,27]. The presence and/or absence of specific mutations can dictate cancer therapy and hence detection of the mutational profile of a given patient is an important step in effective treatment [28,29]. Liver cancer is a dynamic disease which may become more heterogenous. As a result of this heterogeneity, the tumour might include a diverse collection of cells habouring molecular signature with different levels of sensitivity to treatment. Tumor heterogeneity, an effect of genome instability, reduces the efficacy of targeted drugs in personalized therapy, a therapeutic approach where treatments are based on the genome of the diseased individual [30-32].

This study is designed to unveil the oncogenic characteristics of the mutated and non MDM2 gene and the variations that exist in the protein structure characteristics which plays a major and significant role in the design and development of therapy for liver cancer sufferers and to further unmasked the genetic factors and interactions that triggers loss of functions and deviant expressivity of the MDM2 gene.

Materials and Methods

The study location

The study was carried out at the Department of Internal Medicine, Gastroenterology and Hepatology Unit, University of Calabar Teaching Hospital, Calabar, Nigeria from September 2017 to September 2020.

Ethical approval

Ethical approval was obtained from the University of Calabar Teaching Hospital Ethical Review Committee before the commencement of this study.

The study population

A total of 55 participants were recruited for the study; comprising 45 cases from clinically diagnosed liver cancer patients and 10 aged-matched controls who were all Nigerians.

Inclusion criteria

The 45 liver cancer patients (hepatocellular carcinoma, liver cirrhosis) were diagnosed by the clinician based on either triphasic CT scans, positive histologic findings of focal lesions or an elevated alpha feto-protein (AFP) level (greater or equal to 400 ng/ml), combined with at least one positive image on sonography, and/or high-resolution contrast computed tomography. Hepatocellular carcinoma cases that were newly diagnosed, previously untreated (neither chemotherapy nor radiotherapy) and free from any other cancer were included in this study. The absence of hepatocellular carcinoma was established by the absence of focal lesions on the triphasic CT scan and by an elevated alpha-fetoprotein level (400 ng/ml).

Exclusion criteria

In the control, any possible history of hepatocellular carcinoma was excluded and subjects of non-African descendant were not recruited in the study.

Subject enrolment and sample collection

Informed consent was obtained from the patients, guardian or parent before data collection and sample collection was initiated. Detailed medical history, age, race or ethnicity, social history/risk factors like alcohol intake, multiple sex partners/unprotected sex, dietary habits and family history of liver cancer case(s) was collected from the subjects recruited for this study. 2-3 ml of whole blood was collected from both patients and controls, put into EDTA bottle and labeled properly. Blood samples was stored in deep freezer (-20^0c) before onward transportation to the virology and molecular diagnostic units, International Institute for Tropical Agriculture (IITA), Ibadan, Oyo State, Nigeria for molecular analysis.

Genomic DNA extraction/molecular analysis

DNA was extracted from all the blood samples. DNA extraction was carried out according to [33] protocol with some modifications as reported by [34]. About 150µl of whole blood was put into 1.5ml eppendorf tube placed on a rack and 350µl of extraction buffer (Tris base, EDTA, $NaCl_2$ and monothioglycerol) was added to the tubes. Forty micro litre of 20% Sodium dodecyl sulphate (SDS) was then added and the tubes were inverted three to four (3-4) times before they were incubated in a water bath at 65˚c for 10 minutes. Potassium acetate (160 µl of the 5M solution) was added to the tubes. The tubes were inverted two to three (2-3) times and centrifuged at 10,000 rcf for 10 minutes. About 400 µl of the supernatant was carefully transferred into eppendorf tubes and 200 µl of cold isopropanol was then added. The tubes were inverted gently for about 5-6 times to precipitate DNA. The tubes were kept in the freezer (-20˚c) for 10-20 minutes. The tubes were then centrifuged at 10,000 rcf for 10 minutes to sediment the DNA. The supernatant was carefully decanted gently in order to ensure that pellet was not disturbed, 500µl of cold ethanol was added to the pellet to wash the DNA, then centrifuged at 10,000 rcf for 5-10 minutes. The ethanol was decanted and the DNA was air-dried at room temperature until no trace of alcohol was seen in the tubes.

The DNA was re-suspended in 50 µl of TE buffer and stored in the freezer as stock solution. An aliquot of the DNA stock solution was ran on agarose gel electrophoresis to check the DNA quantity. Each sample of genomic DNA of about 4 µl was mixed with 2 µl of loading dye and transferred into the wells of the gel. A voltage of about 110v-120v was applied for about 45 minutes. The samples were scored faint, +, 2+, 3+ depending on the intensity of the bands to indicate the amount of DNA extracted after mounting and viewing in the automated gel documentation system.

Primer selection

Specific set of primer(s) suitable for MDM2 gene were used for the amplification. The amplification of MDM2 gene on exon 1 and intron 1 were carried out using two sets of primers. The forward primer was B-MDM2-309F 5^1-GGGAGTTCAGGGTAAAGG-3^1 and the reverse primer was B-MDM2-309R 5^1-GACCAGCTCAAGAGGAAA-3^1 [34]. The PCR amplified 174 base-pairs of MDM2 gene using the primer above. The reaction was carried out using a total of 25µl reaction,comprising 18.3µl of dH_2O (distilled water), 2.5µl of 10 X buffer, 1µl of dNTPs, 0.5µl of forward primer,0.5µl of reverse primer, 0.2µl of Taq DNA polymerase and 2µl of DNA templates. They denatured at 94^0C for 5 minutes, followed by 40 cycles (94^0C for 30 seconds, 60^0C for 35 seconds, and 720C for 30 seconds). Annealing was performed at 55^0C for 60 seconds, then final extension at 72^0C for 10 minutes.The amplicons of 174 base-pair were visualized by staining the gel with ethidium bromide, after electrophoresis on 2% agarose gel [35].

Purification of amplicons

Amplicons were purified at the Virology and Molecular Diagnostics Unit, International Institute for Tropical Agriculture (IITA), Ibadan, Oyo State, Nigeria using the alcohol based method [36]. Approximately, 21µl of amplicons were put into newly neatly PCR tubes and 52.5µl of 95% ethanol (21µl x 2.5µl) was added to precipitate the amplicons. It was then centrifuged at 12,000 rcf for 10 minutes and gently decanted; remaining the pellets attached to the wall of the tube. Approximately, 500µl of 70% alcohol were added and then centrifuged at 12,000 rcf for five minutes. It was then carefully decanted and the amplicon pellets were air dry at room temperature (25^0C- 28^0C) [37]. Finally, 22µl of distilled water was added to each PCR tube pellets of purified amplicons and stored in deep freezer for further usage. The suspended purified amplicons tubes were wrapped in foil and packed in ice containers for shipment to Inqaba Biotec Laboratory, South Africa.

Sequencing of the genes

Bidirectional sequencing of the purified amplicons was carried out at Inqaba Biotec Laboratory, South Africa. Same set of primers for PCR amplification of the MDM2 gene was used in the bidirectional sequencing of the candidate gene (MDM2 gene).

Bioinformatics analysis of the genes sequences

The nucleotide sequence of the MDM2 gene targeted on exon 1 and intron1 was decoded from the chromatograms using ChromasPro software (www.technelysium.com.au). Multiple alignments were carried out using Clustal W in MEGA 6.06 according to [38] and excluding all the gaps. Phylogenetic analysis was constructed using MEGA 6.06 software [39]. CodonCode Aligner version 6.06 was used to analyze the SNPs in the aligned sequences. NETWORK 4.6.1.1 was used for network analysis of haplotype distribution [40], while DnaSP 5.0 was used to assess the haplotype numbers and their frequencies.Tertiary protein structure was constructed using Phyre2 (polymorphic Phenotyping 2) software and viewed using RasMol molecular graphic tool. The STRING option of expasy. org. an online interactive program was used to determine the oncogenic functional partners of the mutated MDM2 gene of the liver cancer patients.

Statistical analysis

The socio-demographic variables and clinical data were computed and analyzed using Statistical Package for Social Sciences (SPSS) version 20.0. Continuous variables were compared between liver cancer patients and controls using student *t*-test.

Results

Oncogenic and liver function test results of patients

In table 1, the results revealed that out of the 45 patients recruited for the study, 15.1% were HIV positive, 17.7% were diabetic, 31.1% had melanin stool 24.4% had ascitis and 17.7% had hepatic encephalopathy as shown in Table 1. The liver function test carried out on the patients revealed that the albumin was 43.58 ± 9.42 g/dl, creatinine was 136.62 ± 177.98 µmol/l, urea was 4.20 ± 1.88mmol/l, the platelet was 180.74 ± 104.70 $x10^9$ l/cells, the haemoglobin was 11.01 ± 3.36g/dl, the alpha-fetoprotein (AFP) present was 398.8 ± 103.2ng/ml, the aspartate aminotransferase (AST) was 45.65 ± 66.01 µmol/l, alanine transaminase was 31.67 ± 33.37 µmol/l and the international normalized ratio (INR) was 6.4 ± 23.04.

Variable	Total (n = 45)
HIV positive	7 (15.1)
Diabetic cases	8 (17.7)
Melena stool	14(31.1)
Ascitis	11 (24.4)
Hepatic encephalopathy	8 (17.7)
Albumin (g/dl)	43.58 ± 9.42
Creatinine (µmol/l)	136.62 ± 177.98
Urea (mmol/l)	4.20 ± 1.88
Platelet ($x10^9$ l/cells)	180.74 ± 104.70
Hb (g/dl)	11.01 ± 3.36
AFP (ng/ml)	398.8 ± 103.2
AST (µmol/l)	45.65 ± 66.01
ALT (µmol/l)	31.67 ± 33.37
INR	6.4 ± 23.04

Table 1: Oncogenic and liver function test of patients.

AFP: Alpha-feto Protein; ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; INR: International Normalized Ratio; HIV: Human Immuno-Deficiency Virus; Hb: Haemoglobin, Normal range for liver function test:

AFP – 10 ng/mL – 20 ng/mL, AST – 8 µmol/L – 33 µmol/L, ALT- 7 µmol/L -56 µmol/L.

Oncogenic physicochemical characteristics of mutated and non-mutated MDM2 gene isolates

Table 2 presents the results of oncogenic physiochemical properties of the mouse double minute 2 gene isolated from patients with HCC liver cancer and from control group without HCC liver cancer disease. The results showed the variations in physiochemical characteristics in the oncogene among the two isolates. The knowledge of the oncogenic physiochemical characteristics of the MDM2 gene plays a significant role in the design and development of therapeutic targets for the protein targets of the isolates. The instability index, Guanine - cytosine contents and aliphatic index all fall below the recommended standard for genetic and thermal stability indicating that the isolates are genetically and thermally unstable with very short half-life and high extinction coefficients.

- **Molecular weight**: The results of variations in molecular weight of the MDM2 gene revealed that the treatment group MDM2 gene has mutated through deletion leading to a reduction in grams per molecule and number of nucleotides.
- **Total size of MDM2 gene**: As a result of mutation, the total size of the MDM2 gene reduced by 75 bps in size compared to the control group.
- **Coding region of the MDM2 gene**: The coding region or exon portion of the MDM2 gene which is the region of the gene expression showed a shortened coding region for the patient (35 - 384) thus giving way for the proto-oncogenes to obstruct the expression and functionality of the MDM2 gene but this was not the case with the control group which gene occupied the normal motif for the coding region from 32 - 395.
- **Total number of amino acids**: The total number of amino acids was also reduced in the patients due to mutations revealing only 479 KDa compared the 504 KDa obtained from the control group. A total of 25 KDa total amino acids was lost due to deletion mutations.
- **Aliphatic index**: The aliphatic index is a measure of stability of the MDM2 gene against forces
- of mutations by surrounding the gene with aliphatic side chains amino acids of leucine, isoleucine, valine and alanine as the building blocks amino acid. The control group had a measure of genetic and thermal stability more than the patients. This subsequently resulted to the mutations in the patients MDM2 gene leading to liver cancer.

- **Instability index** of the MDM2 gene: instability index measures the genetic and thermal stability of a gene with instability index of 1 - 38% revealing more stability and 39 - 100% revealing increasing instability. For the MDM2 gene from the patients and control groups both showed genetic and thermal instability from the results in table 2.
- **Guanine - Cytosine (G-C) content of MDM2 gene:** The results of the guanine - cytosine contents of the MDM2 gene for the patient and control group further revealed that both genes are thermally and genetically unstable as a percentage of greater or equal to 46% and above indicate increasing stability while a less than or equal to 45% and below indicates decreasing stability of the MDM2 gene as evident from the MDM2 genes in patients and control.
- **Extinction coefficient of the MDM2 gene:** This characteristic measures the expressivity and functionality of the MDM2 gene when exposed to light (UV) or other associated radiations. The results indicates a high extinction coefficients for the MDM2 gene for both patients and control group suggesting that mutations in the MDM2 gene in the patients is attributable to exposure of MDM2 to high UV leading to mutations of the gene.
- **Hydropathicity of MDM2 gene:** This characteristic measures the hydrophobic and hydrophilic attractiveness of the MDM2 gene when exposed to solutes which also break the bond and atoms binding their molecules together. Negative hydropathicity value indicates hydrophobicity (less attractive to water and other solutes) while positive hydropathicity indicates hydrophilicity (more attracted to water and other solutes).

S/N	Oncogenic Characteristics/ properties of MDM2 gene	Patients (MDM2 gene)	Control (MDM2 gene)
1	Molecular weight of the SARS-COV 2 (g/mol)	55990.80	58924.31
2	Total Size of the MDM2 gene (bps)	1491	1566
3	Coding region of the MDM2-gene	35 - 384	32 - 395
4	Start codons of the MDM2 gene	35	32
5	End codon of MDM2 gene	384	395
5	Total number of amino acids in MDM2 gene	479	504
6	Aliphatic index (side chain amino acids) (%)	65.63	67.72
7	Alanine (%)	3.0	3.1
8	Valine (%)	6.4	6.5
9	Isoleucine (%)	4.0	4.0
10	Leucine (%)	7.2	7.9
11	Instability index (%)	58.71	57.56
12	Extinction/attenuation coefficients of the MDM2 gene (m/M^3)	0.766	1.027
13	Hydropathocity of MDM2 gene	-0.739	-0.739
14	Half- life of MDM2 gene (hours)	30	30
15	G-C contents of the MDM2 gene (%)	38.51	38.78

Table 2: Oncogenic Characteristics of MDM2 gene causing Liver Cancer isolated from patients and control group.

MDM2 gene oncology (MDM2 gene interactions with associated functional molecular partners).

MDM2 is mouse double minute 2 gene also known as E3 ubiquitin-protein ligase Mdm2. E3 ubiquitin-protein ligase mediates ubiquitination of p53/TP53, leading to its degradation by the proteasome. MDM2 Inhibits p53/TP53- and p73/TP73-mediated cell cycle arrest and apoptosis by binding its transcriptional activation domain. It also acts as an ubiquitin ligase E3 toward itself and ARRB1. It Permits the nuclear export of p53/TP53 and promotes proteasome-dependent ubiquitin-independent degradation of retinoblastoma RB1 protein thereby inhibiting DAXX-mediated apoptosis by inducing its ubiquitination and degradation. See Figure 1 for interactions with functional partners.

EP300 is histone acetyltransferase p300. The gene functions as histone acetyltransferase and regulates transcription via chromatin remodeling. It also acetylates all four core histones in the nucleosomes. Histone acetylation gives an epigenetic

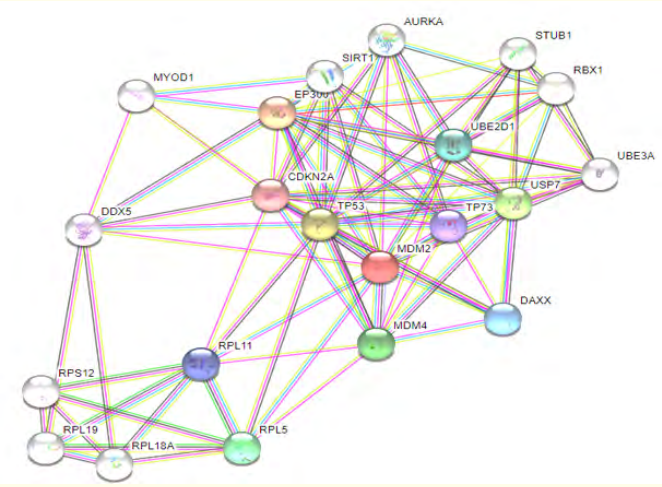

Figure 1: Showing MDM2 gene interaction (oncology) with other molecular functional partners of protein genes in Liver cells.

tag for transcriptional activation in the cells. It mediates cAMP-gene regulation by binding specifically to phosphorylated CREB protein. It further mediates the acetylation of histone H3 at 'Lys-122' (H3K122ac), a modification that localizes at the surface of the histone octamer and stimulates transcription, possibly by promoting nucleosome instability. See Figure 1 for interactions with other functional partners.

TP53 is cellular tumor antigen p53. It acts as a tumor suppressor in many tumor types. It induces growth arrest or apoptosis depending on the physiological circumstances and cell type. It involved in cell cycle regulation as a trans-activator that acts to negatively regulate cell division by controlling the set of genes required for this process. One of the activated genes is an inhibitor of cyclin-dependent kinases. Apoptosis induction seems to be mediated either by stimulation of BAX and FAS antigen expression, or by repression of Bcl-2 expression. See Figure 1 for interactions with other functional partners.

USPT is Ubiquitin carboxyl-terminal hydrolase 7. The hydrolase that deubiquitinates target proteins such as FOXO4, p53/TP53, MDM2, ERCC6, DNMT1, UHRF1, PTEN and DAXX (Figure 1). Together with DAXX, it prevents MDM2 self-ubiquitination and enhances the E3 ligase activity of MDM2 towards p53/TP53, thereby promoting p53/TP53 ubiquitination and proteasomal degradation. Deubiquitination of p53/TP53, prevents the degradation of p53/TP53, and enhances p53/TP53- dependent transcription regulation, cell growth repression, apoptosis and strongly stabilizes p53/TP53. See Figure 1 for interactions with other functional partners.

MDM4 is Protein Mdm4. it inhibits p53/TP53- and TP73/p73-mediated cell cycle arrest and apoptosis by binding its transcriptional activation domain. It inhibits the degradation of MDM2 and reverses MDM2-targeted degradation of TP53 while maintaining suppression of TP53 transactivation and apoptotic functions. See Figure 1 for interactions with other functional partners.

TP73 is Tumor protein p73 which participates in the apoptotic response to DNA damage. The isoforms containing the transactivation domain are pro-apoptotic while isoforms lacking the domain are anti-apoptotic and block the function of p53 and transactivating p73 isoforms. It's also a tumor suppressor protein. See Figure 1 for interactions with other functional partners.

RPL11 is 60S ribosomal protein L11. This component of the ribosome is a large ribonucleoprotein complex responsible for the synthesis of proteins in the liver cell. The small ribosomal subunit (SSU) binds messenger RNAs (mRNAs) and translates the encoded message by selecting cognate aminoacyl- transfer RNA (tRNA) molecules. The large subunit (LSU) contains the ribosomal catalytic site known as the peptidyl transferase center (PTC), which catalyzes the formation of peptide bonds, thereby polymerizing the amino acids delivered by tRNAs into a polypeptide chain. See Figure 1 for interactions with other functional partners.

CDKN2A is Cyclin-dependent kinase inhibitor 2A; this protein acts as a negative regulator of the proliferation of normal liver cells by interacting strongly with CDK4 and CDK6. This inhibits their ability to interact with cyclins D and to phosphorylate the retinoblastoma proteins. See Figure 1 for interactions with other functional partners.

Secondary protein structure characteristics of MDM2 gene causing Liver Cancer

The MDM2 protein structure revealing the secondary protein structure characteristics of the MDM2 gene as presented in Table

2. The various strands of the MDM2 protein structure are revealed by the different secondary protein structure folding as Alpha helix (pink), random coil (blue), extended strand (green) and beta sheet (yellow). Plate 1b shows that controls (49 as case study) having similar protein folding with patient. The variations in the secondary protein structure folding of the treatment and control cases as presented in Table 2 and plates. 1a and 1b is a reflection of the strengths of the MDM2 gene with reference to the building blocks or helices which forms the skeletal framework and background dimensions of the gene. The variations in the secondary protein structure in MDM2 gene in the patients and controls further reveals that mutation has taken place in the gene.

S/N	Sec. Protein Characteristics	Patient (MDM2 gene)	Control (MDM2 gene)
1	Alpha Helix (%) (Pink)	17.50	17.60
2	Beta Sheet (%) (Yellow)	32.40	32.70
3	Extended Strand (%) (Green)	24.50	24.20
4	Random Coil (%) (Blue)	25.60	25.50

Table 3: Percentage distribution of secondary protein folding characteristics of MDM2 gene in liver cancer patient and control group.

Plate 1a: Ribbon protein structure of MDM2 gene (Patient) showing distribution of secondary protein characteristics.

Plate 1b: Ribbon protein structure of MDM2 gene (Control) showing distribution of secondary protein characteristics.

Tertiary protein structure variations in MDM2 gene causing Liver cancer among patient and control group

The 3 -D tertiary protein folding structure can be model and optimize to provide the side chains and N - terminal amino acids as sites for targets by drugs and other small molecules (ligands). With the development of the structural 3 - D model of the coding regions of the MDM2 gene, it is possible to develop therapy for the Liver cancer using the structure - based drug design, a computer aided drug design (CADD, SBDD) approaches. The tertiary protein structures as shown in plates 2a and 2b for the patients and control groups revealed variations in the mutations of the MDM2 gene structures which appears very distinct in the mouse like and double folding nature of the mouse double minute 2 gene (Figure 2a and 2b).

The stability of a MDM2 protein gene also determines its reactivity with drugs and other small molecules / compounds in terms of rate of reactions, complex formations, dissociation and dissolution in specific solvents and media. Hence, the mutations of the MDM2 gene in the patient is responsible for the changes and variations noticeable with the control as shown in the figures 2a and 2b and is responsible for the MDM2 gene expression of tumour symptoms associated with liver cancer in the patient.

Figure 2ai, 2aii and 2aiii: Ahowing variations in tertiary protein folding structure of MDM2 gene (patient).

Figure 2bi, 2bii and 2biii: Showing variations in tertiary protein folding structure of MDM2 gene (Control).

Discussion

The Swiss model option of expasy.org was used to validate the tertiary protein structures and the difference in protein structures which may be attributed to the MDM2 gene mutations. This is because wild type and mutant type of genes are different in their conformation and protein folding potentials thereby leading to a change in gene function and expression. The resulting SNPs are evidence of mutation in the nucleotide bases of MDM2 gene and may be useful to inform future studies on liver cancer.

The tertiary protein structures of patients and controls group were relatively different, especially among individuals that have more than one type of mutations. This difference in the folding in the protein structures may be attributed to the MDM2 gene mutations. This finding was similar to [41,42] which reported that wild type and mutant type of genes were different in their conformation and protein folding. It is possible that the mutations may have resulted in incorrect solvent accessible surface characteristics in the mutant types of MDM2 genes, thereby changing protein structures, properties and functions.

The secondary folding structure characteristics of a protein is the regular repeating organization of the polypeptide heavy and light chains and represents the backbone of the organism framework and stability. This characteristic of the coronaviruses determines to a large extent, the reactivity of the viral protein coat, the thermal as well as the genetic stability of the MDM2 protein gene [43,44]. Protein coat dominated by alpha helices will present a more resilient pathway for reactions with drugs than those dominated by other secondary protein structures.

Globular protein stability decreases with increasing index volume occupied by the aliphatic side chain. As the volume occupied by the aliphatic side chain consisting primarily of the amino acids, alanine, valine, isoleucine and leucine increases, the thermostability of the MDM2 gene decreases as the amino acids are synthesized with time. The aliphatic (side chains) index acts as barrier that needs to be broken for reactivity with drugs, targets, small molecules and compounds to take place [45-47]. The aliphatic index secures the protein stability and selective reactions with drug targets and protein molecules. The measure of the aliphatic index of a protein is a positive factor towards increasing the thermostability of the globular proteins of the viruses [48-50].

The area of molecular epidemiology focuses on using the genetic sequences of pathogens to understand patterns of transmission and spread. MDM2 mutate very quickly when predisposed to risk factors of HCC and accumulate changes during the process of transmission from one infected individual to another.

Conclusion

The results revealed the oncogenic characteristics and MDM2 gene oncology for patients suffering from liver cancer. The study identified distribution of secondary protein structure characteristics as well as the variations in tertiary protein structure characteristics of MDM2 gene responsible for cancer of the liver. The study unmasked the existing relationships and interactions between the mutated MDM2 gene and other functional protein partners which can be harnessed to design a therapeutic pathway for the genetic related disease of the liver. It was thus concluded that knowledge of the oncogenic characteristics of the mutated and non-mutated MDM2 gene, the functional protein partners and MDM2 gene oncology and the variations that exists in the protein structure characteristics can play a major and significant role in the design and development of therapy for liver cancer disease

and help to unmasked the genetic factors and interactions that triggers the loss of functions and deviant expressivity of the MDM2 gene among patients. Hence, the findings of this study serve as baseline information for further studies on the genetic etiology and therapeutic advancement for the liver associated disease.

Statements and Declarations

Authors have disclosed non-financial interests that are directly or indirectly related to this work submitted for publication. No funding was received for the work.

Competing Interests

The authors have declared no competing interest in the manuscript.

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