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Original Research

Design of Novel Putative Peptide fragments derived from exons of p53 isoform g and p53 promoter region ORF sequence targeting HDM2/MDM2-p53 interaction *

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Abstract

p53 is a potent endogenous tumour suppressor agent which guards against DNA damage and prevents transformation of normal cells into malignant cells. In most forms of cancers except malignant melanoma, mutation occurs in p53 gene rendering it dysfunctional or suppressed. This research attempts to design novel putative bioactive oligopeptides from tumour suppressor p53 gene sequences using its promoter sequence and deriving several primer-based oligomers from the exons of p53 gene. p53 induces cells to undergo repair or apoptosis in response to DNA-damage induced stress and thus acts as the Warden of the Cell. We devise some novel methods to derive our *de novo* sequences from the exons of this gene and report discovery of two new oligopeptides such as HRGRES and PAAPA (P_7TA_7) peptide being two putative oligopeptides.

Keywords: *p53, oligopeptide, peptidomimetics, PAAPA (P_7TA_7) peptide*

∗. This research is a part of multistep endeavour aimed to understand and discover novel therapeutic modalities targeting various aspect of cancer genomics and genomic pathology. The author thanks the staff of the Central Library, Department of Pharmaceutical Sciences of Andhra University for their continued help with the necessary materials and periodicals.

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1 Introduction

Cancer cells have a unique property of avoiding apoptosis—cell suicide. The apoptosis pathway is as well disrupted in most human cancers. One of the key players in cell suicide pathway is tumour protein p53 (Soengas et al., 1999) whose activation following DNA damage sends a death signal to the cell. This agent–p53 has become a topic of intense research and investigation (Oren 2003, Bourdon et al., 2005) ever since its discovery as a protein interacting with the oncogenic T antigen from SV40 virus (De Leo et al., 1979, Kress et al., 1979). It has been marked as a prominent tumour suppressor protein having the most frequent single gene impact on the genesis and suppression of human cancers (Bourdon et. Al 2005). Being a pleiotropic transcription factor, p53 helps to maintain genetic stability which prevents transformation of normal cells from being cancerous (Khouri and Bourdon 2010). Therefore, p53 suppresses cancer development (Gomes and Espinosa 2010). The mechanism by which p53 induces cell suicide is complex and involves a cascade of signalling peptides which includes both upstream and downstream regulators. Besides, p53 is considered to be one of the most important tumour suppressor proteins which play an important role in preventing genome mutation. This gene, cellular tumour antigen p53—also known as BCC7, LFS1 and TRp53 or simply p53 is found to be mutated in almost 50% of all cancers (Zmijewski, Lane and Bourdon 2006, Muller and Vousden 2013, Shen et. al., 2014). The TP_{53} suppressor gene is sometimes referred to as the "Warden of the cell" which guards genome integrity in the living cells. The gene which encodes p53 is located on the short arm of chromosome 17 (McBride, Merry and Givol 1986). Research indicates that p53 has a definite role to play in restraining DNA exchange between imperfectly homologous sequences and that it suppresses tumorigenic genome rearrangements (Akyüz et al., 2002). It has also been postulated that p53 is directly involved in DNA repair mechanism (Akyüz et al., 2002). Genomic integrity is essential for cell growth and differentiation. p53 is a protector of genomic integrity. Transcriptional activation of p53 tumour suppressor gene provides a protective mechanism against DNA damage (Reisman 2013). In over 50% of all types of human cancers, p53 gene mutation and dysfunction have been found to play a major role in inactivation, silencing and dominant negative inhibition of wild-type human p53 (Shen et. al. 2014). Since DNA repair is central towards maintaining cell integrity, it is for the reason that errors in repair mechanism could give rise to mutations which could result in translocations, deletions, duplications, and expansions. On the other hand, translocations, deletions, duplications and expansions lead to DNA damage. Mutagenic effects resulting in faulty DNA repair mechanism leads to tumour genesis and progression. In response to such damaging injuries to DNA, tumour suppressor p53 protein comes to play significant role in inducing transient cell cycle arrest by transcriptional transactivation of target genes to allow repair or induce apoptosis. In

fact, evidence strongly suggests that mutation or other mechanisms that inactivate p53 transcriptional factor leads to tumorigenesis (Hong et al., 2014). Mutations have been observed within the DNA-binding domain of p53 as "mutational hotspots" (Pfeifer and Besaratinia 2009). The nature of mutations that alter p53 functionality are mostly missense mutations (Muller and Vousden 2013, Vogelstein and Levine 2000) although frameshift mutations and silent mutations do occur to a much lesser extent (Muller and Vousden 2013, Vogelstein and Levine 2000). Experiments with transgenic zebrafish have shown that p53 loss of function is often responsible for the genesis of cancer (Storer and Zon 2010). Another endogenous tumour suppressor agent is retinoblastoma protein (pRB) which is also found to be mutated in human tumours (Goodrich and Lee 1993). Normally, DNA damage, hypoxic stress, oncogene activation and nutrient deprivation induce activation of p53 (Hong et al., 2014, Reisman 2013). Activation of p53 results in induction of p^{21} following DNA damage which inhibits the activities of Cdk4 and Cdk2 (He et al., 2005). The endogenous levels of p53 are generally low but they are found to rise in response to cellular stresses such as DNA damage. p53 levels are kept low due to its rapid degradation by ubiquitin-dependent proteolysis. If DNA damage is significant, p53 promotes the cell to undergo apoptosis (Halazonetis, Gorgoulis, and Bartek 2008, Bartek and Lukas 2007). But if p53 gene is itself damaged due to mutation, it fails to promote the cell to undergo apoptosis. On the other hand, over-expression of p53 can lead to premature aging (Vousden and Lane 2007). Mutations within Tp53 gene gives rise to mutated isoforms which may render the protein non-functional. This paper specifically aims to develop a novel putative bioactive chemotherapeutic oligopeptide fusion compound based on HDM2 (MDM2)-p53 interaction proteomics which targets to break the HDM2-p53 interaction so that it leads to accumulation of p53 in the cell. Furthermore, the attempt is to generate p53-promoter specific ligands which could bind to specific sequence region in its promoter region and could possibly affect positively to stimulate its expression or alter its level of activities. In fact, models of interventions affecting binding of cytoplasmic domain with the regulatory unit of a gene have become hot targets to develop novel therapeutic regimens which could alter gene expression and functionalities. Vigorous efforts are on the way to develop compounds which could target interactions between genes and their responsive elements. One important pathway which has become critical in the modulation of intercellular p53 gene levels is the interaction between HDM2/MDM2 and p53 protein. MDM2 is a natural antagonist of p53 which binds to it and suppresses its activity as a transcription factor (Chi et al., 2005). In most forms of cancer, p53 levels are found to be low or the gene encoding p43 protein is found to be mutated. Viable therapeutic option for cancer could be attempted by gene therapy methods to introduce specifically designed oligoprimer sequences which might rectify such mutations in p53 gene. Also, pharmacological reactivation of p53 by MDM2 inhibitors is a viable treatment option of cancer (Vassilev 2007, Ding et al., 2006). It is for the reason that p53is a powerful antitumour protein most frequently inactivated by mutations or deletions in cancer (Vassilev 2007), and this protein, being further broken down by MDM2 in the cell, could be reactivated by selective inhibition of HDM2/MDM2 gene to augment the levels of p53 in the cell. Several approaches have

been underway as novel drug design modalities targeting specific factors in cell cycle dynamics to treat cancer; for instance, bioactive compounds are being designed that blocks the attachment of ubiquitin to p53 (Hoeller and Dikic 2009), or blocking the interaction of MDM2 and p53 (Wang et al., 2012, Nag et. al., 2014). Also, drugs are being designed to stimulate MI-63 which binds to HDM2 and prevents its interaction with cellular p53 (Ding et al., 2006). This research develops a novel method that helps to design putative bioactive peptides by remodelling oligopeptides derived from exon-specific oligoprimer sequences of p53 to restore its normal endogenous levels in cells. The putative bioactive compound so designed is meant to block the interaction between HDM2/MDM2 and p53 and further act as a specific to augment p53 expression. The activity of p53 as a transcription factor is suppressed when it is bound to HDM2/MDM2. Furthermore, HDM2/MDM2 promotes rapid degradation of p53 which leads to its depletion in the cell (Chi et al., 2005). We attempt to model p53 exon-specific biomimetic oligoprimer-derived structure-based novel peptides as potential putative agents that aim to interact and affect restoration of endogenous levels of p53 protein in cancers by preventing its degradation and/or inducing its reactivation. Several researches have explored and are still exploring *de novo* methods in designing ubiquitin-specific USP_7 (HAUSP) protease that cleaves ubiquitin off p53 (Reverdy et al., 2012). Attempts have been in the line to develop drugs that block the action of HDM2 in human cancers (Issaeva et al., 2004), or drugs that stimulate M1-63 for it binds to MDM2 and inactivates it. In fact, understanding and targeting cancer genes and the pathways they control (Vogelstein and Kinzler 2004) is yielding novel methods of cancer therapeutic regimens (Borghouts, Kunz, and Groner 2005) for instance, ligand-targeted therapeutics (Allen 2002), and use of monoclonal antibodies to treat hematologic malignancies (Reff, Hariharan, and Braslawsky 2002) among others. For example, simple three-amino acid peptides like The RGD (Arg-Gly-Asp) and NGR (Asn-Gly-Arg) represent first generation homing peptides used in antitumor therapeutics (Zitzmann, Ehemann, and Schwab 2002, Thundimadathil 2012). For a detailed review of peptide-based cancer therapeutics, See Aina et. al. (2002), Thundimadathil (2012), Xiao et al. (2015).

2 Materials and Method

We explore the possibilities of designing novel bioactive peptide-based agents which may possess putative bioactivities targeted towards cell cycle signalling pathways and to design potential reactivators of tumour suppressor genes. For our research, we derived p53 exon-specific primers to design putative oligopeptides that might have therapeutic value by their modification, structural alteration and by addition of other functional groups to the designed oligopeptides. This is attempted to mimic the existing peptide chemotherapeutic agents in use for the treatment of cancer and further analyze how the structure correlates to its biological activities. Among many new therapeutic modules, peptide-based chemotherapy has been of great interest due to their low molecular weight, low toxicity to normal cells and tissues, and their

specific ability to target tumour cells (Xiao et al., 2015).

2.1 Selection of p53 Sequences

For this research, we perform our analysis on p53 nucleotide sequences; these include Homo sapiens tumor protein p53 (TP53) transcript variant 2 mRNA with an accession number NM_001276761.1 derived from NCBI having Reference Sequence ID: NM_001276761, and Homo sapiens Mdm2 p53 binding protein homolog (mouse) mRNA cDNA clone, partial CDS GenBank: BC152384.1, and CDS TP_{53} cellular tumour antigen isoforms 'g'. We perform rigorous analysis on these sequences using genomics and bioinformatics tools and methods to decipher unique features present in these transcripts. Initially, we perform analysis of human p53 gene encoded by TPp53 transcript variant 2 mRNA. We generate several open reading frame (ORF) sets using FASTA and Genbank formats sourced from NCBI. BLAST search has been accomplished to match the results with our output following methods of pairwise and multiple alignment techniques of genes to detect any heterogeneity among them. Although a complete six-frame translation data of the sequence have been done, we did not seek to search for unique proteins and protein binding domains in this paper. In the translated frame of the protein sequence, we searched for Amidation sites, Protein Kinase C phosphorylation sites, and N-glycosylation sites using CLC Main Workbence 8.0. Detection of CpG islands and microsatellites and STS has been performed as well (not reported). The results of all these analyses were assembled and analyzed using several proteomics software. Following this, we performed oligo analysis and generated exon-specific primer probes derived from primer polypeptides of p53 exons. Translation-specific data was used to perform statistical analysis to generate probable therapeutic oligopeptides and elucidate in detail their chemical structures—including stereochemistry and enantiometric analysis. Oligopeptides thus derived were modelled computationally using chemical structure elucidation software. Following exon-specific oligoprimer design, we developed a peptide and designed p53 promoter-specific sequence as a putative primer-binding probe that is aimed to mimic signals within the promoter region of the p53 gene. This is attempted to stress the mechanism of selection of promoters for initiation of transcriptional activation of this gene. There are various signal subunits (peptides) which help to initiate transcription. However, the promoter region sequence of p53 gene is unique. Finally, we fuse both these two peptide compounds to derive a single putative peptide with the aim of obtaining an optimal configuration that mimics the transcription initiation signal. But since the attempt is putative there may be several drawbacks, yet with continued modification and optimization, such a putative compound may be highly promising to augment the potential development of a lead compound. Therefore, *de novo* design of a novel putative fusion compound has been undertaken using side chain fragments and heterocyclic parent structures to fuse these with the oligopeptide to derive an entirely new chemical structure but with tentative biological functions. We have not performed any theoretical QSAR study in detail, although detailed QSAR studies may be attempted in future research.

Nevertheless, we generated one fusion compound to elucidate its bioactivity and possible chemotherapeutic activities to derive the most relevant compound of interest assumed to possess some pharmacological effects.

2.2 Analysis of p53 gene

For our research, we obtained the transcript variant 2 mRNA of p53 gene from NCBI Genbank. This p53 gene was initially considered as a tumour inducing oncogene (Lamb and Crawford 1986, Nigro et al., 1989). Nevertheless, later investigations proved this gene to be a tumour suppressor agent based on the evidences that called the prior assumptions in question; experiments had failed to determine that the wild type of p53 gene can transform cells in culture (Finlay et al., 1988). Therefore, this gene was classified under representative tumour suppressor genes which also include several other co-repressors or suppressors, e.g., rB protein, p16 (INK^{4a}), ARF, ATM, BRCA1&2, CHK2, APC (See Charles Sherr 2004 for a detailed review). The transcript variant TP53 gene which encodes for tumor suppressor protein p53 is 2588 base pair (bp) long with a molecular weight of 1599.12 kDa and having a GC content of 53.4%. This gene has several exons which encodes for several isoforms including p53 isoform g which is encoded by this transcript variant 2 mRNA. The protein p53encoded by this gene has several transcriptional activation, DNA-binding and oligomerization domains that are excellent therapeutic targets. We have performed sequence analysis to search for exons and ORFs within the p53 gene and isoform g of p53 tumour suppressor gene and found three ORFs and eight exons in the gene sequence. Exons are the regions in the DNA that codes for proteins and are the regions of an mRNA that are retained after splicing. We report nucleotide sequence statistics (NSS) of 8 key p53 exons in the data section of the appendix.

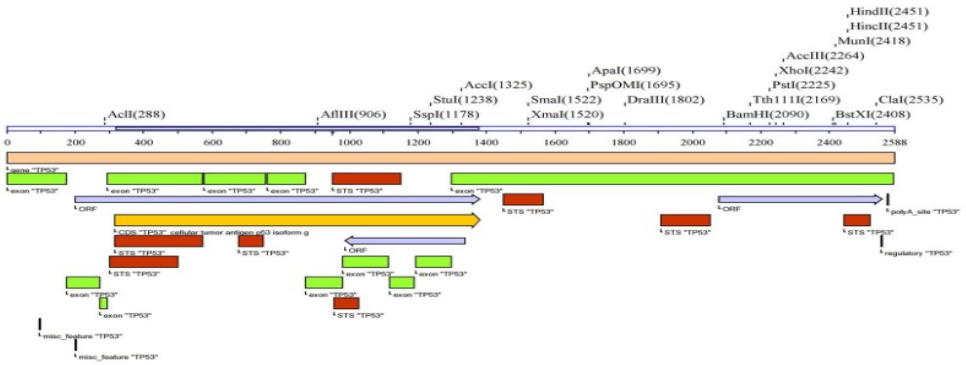

Figure 1. The graphic representation of the p53 gene depicting several exons and the ORFs.

Translation of the sequences including the exons and the ORFs are provided as well, and we have analyzed in detail the translated protein products encoded by the gene fragments. There are numerous Sequence Tagged Regions (STS) found as microsatellites throughout the p53 gene. The ORF region (2080-2553) which codes for 158 amino acids has a unique distribution of T rich poly-thiamine nucleotide

repeats between regions 2125-2163. This region of the ORF with 39 bp has a very low GC% content and a high Thymine content coding for amino acid phenylalanine (F). We examined whether if this poly-T STS site has any biological significance or if it is a conserved region among other tumour suppressor genes like for instance, retinoblastoma protein rB, $p16^{INK4a}$, $p14^{ARF}$. We derived protein translations of these exons following which we designed oligomers and primers based on DNA sequences of all the exons of p53 gene. The figure below represents the restriction map along with circular form of the gene p53.

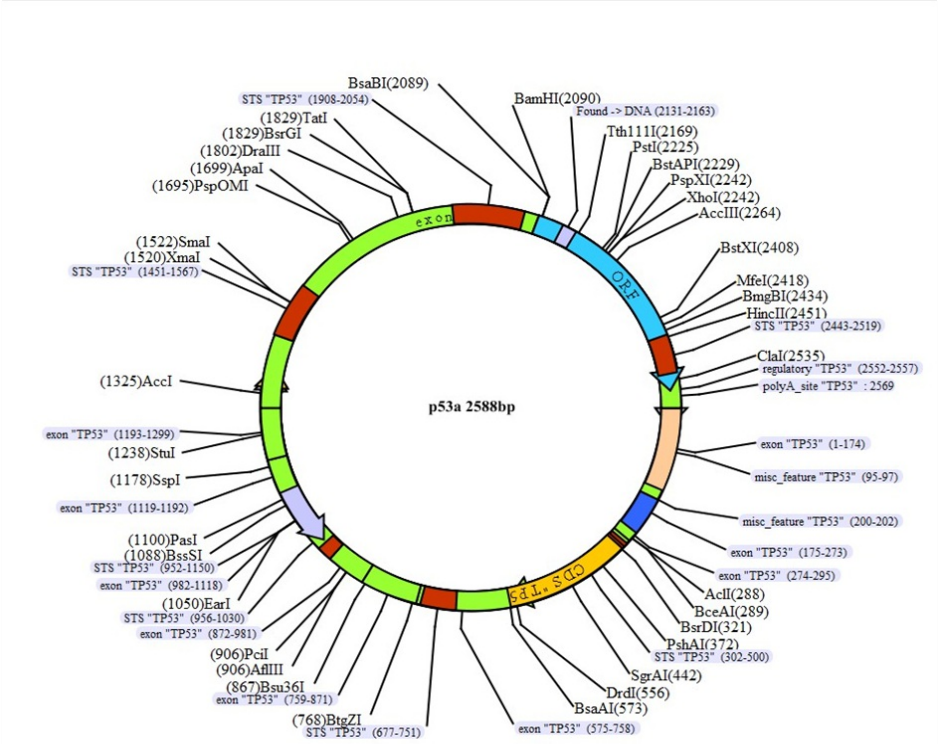

Figure 2. The circular view of the p53 gene with its restriction endonuclease sites.

3 Results

We derived from our analysis of the p53 gene that yielded several ORFs and exons from which we have designed several primers. The exons derived insofar have been marked and their general characteristics annotated (See). The nucleotide sequences of all the exons have been translated into their amino acid and protein products and thus analyzed. The region 1-174 of p53 which is exon 1 has a length of 174′ bp with GC content of 58.05%. The exon 2 region 175-273 has a length of 99′ bp having GC% content 56.57%. Exon 3 at the region 296-574 has a length of 279′ bp with a GC% content of 59.86%. The region 578-758 which is exon 4 is 184′ bp long and has a GC% content of 61.96%. The region 759-871 is tagged as exon 5 which has

a length of 113′ bp with a GC% content of 51.33%. The region 872-981 is exon 6 which is 110′ bp in length and has a GC% content of 53.64%. Exon 7 is marked by the region 982-1118 which is 137′ bp in length and has a GC% content of 59.12%. Finally, the longest exon insofar within the p53 gene is exon 8 which is 1289′ bp long and has a GC% content of 48.95%. This region has some unique features; for, it has unusually high dinucleotide contents with TT repeat units amounting to 147 dinucleotides; between the regions 2125-2163, which is 39 bp in length, the sequence reads as follows: TTTCTTTTTTCTTTTTTTTTTTTTTTTTCTTTTTCTTT with a translated frame of peptide sequence: FLFSFFFFFFFF. In fact, this particular region has a very high concentration of amino acid phenylalanine (F). The translated frame of all the exons with their protein contents have been analyzed following which, we performed statistical computations on the exon nucleotides to identify unique correlations and features specific to each exons with respect to their lengths. Oligomer analysis on each specific primer products designed from the respective exons have been performed, translated and denoted. We designed several primers from exon 1 that include CTCCCATGTGCTCAAGACTG, CCCTCCCATGTGCTCAAGAC, and TCCCATGTGCTCAAGACT respectively, using different PCR primer tools. These are all sense and forward primers. Translation products of these primers/oligomers have been employed for composite ingredients to design and structure novel bioactive peptide agents as possible therapeutic molecules. We have reduced the amplicon length in such occasions where the length of the exon is relatively short. Such a constraint, however, reduces primer quality which may contain self-dimmers, runs and repeats but no hairpins. Following translation of all six frames of the primer CCCTCCCATGTGCTCAAGAC from exon 1, we derived and report its corresponding translated product as PSHVLK from first reading frame. However, we restrict our analysis to one frame translation products for each primer to derive composite elements for design of bioactive peptide compounds. From exon 2, we designed a primer AGCCGCAGTCAGATCCTA and derived an oligopeptide SRSQIL as a prospective peptide. Exon 3 yields a sense primer CGGACGATATTGAACAATGG and its respective translated peptide chain is RTILNN. We derived amino acid sequence PALNKM from the primer CCTGCCCTCAACAAGATG of exon 4. Exon 5 yields a primer and its corresponding peptide translation product as CCTCCTCAGCATCTTATCC and PPQHLI respectively. Exon 6 yields the primer GTTGGCTCTGACTGTACC with its corresponding translated oligopeptide product VGSDCT (Cellular tumour antigen p53). We derived from exon 7 a primer CACAGAGGAAGAGAATCTCC with its corresponding translated frame of oligopeptide as HRGRES. For exon 8, we designed a reverse antisense primer CTTCTGACGCACACCTATT yielding its oligopeptide sequence as LLTHTY. We computed the theoretical pI and aliphatic index of all the derived oligopeptides from the translated primer sequences designed from all of the 8 exons so far. Of all these oligopeptides mentioned and which so far exists in searchable chemical databases (Reactome, Chemspider, Pubchem and eMolecules), we discovered a novel 6 aa oligopeptide HRGRES with a chemical formula [C28H48N14O10] having a theoretical pI of 9.61 , aliphatic index of 0.0 and having a molecular weight of [740.775] gr/mol. The aliphatic index is defined as the relative volume of aliphatic side chain most observable in amino acids alanine, valine,

isoleucine, and leucine (Gasteigar et al., 2005). Higher aliphatic index of a (globular) protein indicates that it is more thermostable. The composition of this formula is given as: C(45.40%) H(6.53%)N(26.47%)O(21.60%). The structure contains one imidazole ring. The structure of the formula has been search intensively and the formula has not been found matching to the existing compounds in conventional chemical databases. The unique formula is drawn and its InChI has been given below:

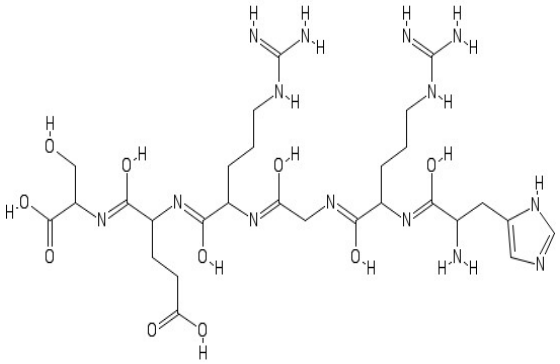

Figure 3. The formula of the novel putative oligopeptide HRGRES ($C_{28}H_{48}N_{14}O_{10}$).

InChI=1S/C28H48N14O10/c29-15(9-14-10-34-13-38-14)22(47)40-16(3-1-7-35-27(30)31)23(48)37-11-20(44)39-17(4-2-8-36-28(32)33)24(49)41-18(5-6-21(45)46)25(50)42-19(12-43)26(51)52/h10,13,15-19,43H,1-9,11-12,29H2,(H,34,38)(H,37,48)(H,39,44)(H,40,47)(H,41,49)(H,42,50)(H,45,46)(H,51,52)(H4,30,31,35)(H4,32,33,36)

This basic oligopeptide is novel, in the sense that the formula does not exist insofar as searchable databases are concerned. However, on query, there is an interresidue similarity found by comparing this oligopeptide with Leucine-rich flightless interacting protein 2 having amino acid sequence DVSGSHRGRESISRRR with protein ID: LRRF2_XENLA (UniprotKB/Swissprot) derived from Xenopus laevis (African clawed frog). Since our oligopeptide does not contain Trp, Tyr or Cys residues, it should not be visible by UV spectrophotometry. The estimated half-life of this peptide is 3.5 hrs, as computed from *Expasy Proparam* program. The half-life of protein depends on the presence of N-terminal amino acids which thus determines its overall stability (Tobias et al., 1991). It is the N-terminal amino acid which simply determines its half-life. This protein is classified as stable. This particular oligopeptide- H*R*G*R*ES contains two arginine residues and we stress the importance of arginine-rich oligopeptides for their important role in the functioning of critical tumour suppressor gene like p53 (Tsuber et al., 2017) Mutations in cancers causing a net gain of histidine, cysteine, and tryptophan at the expense of arginine may be a factor for transition of a normal cell into a malignant cell (Tsuber et al., 2017). We concentrate on this new formula to design a novel bioactive therapeutic peptide as a putative test molecule and a substrate for a fusion compound with another putative peptide that we have designed do far. It is assumed that this peptide possesses some novel biological activity. The constituent of this oligopeptide is a 6aa chain with one acidic amino acid-glutamic acid, and two basic amino acids—arginine (ARG).

3.1 PAAPA peptide (P_7TA_7)

p53 belongs to a family of genes composed of p53, p63 and p73 (Khouri and Bourdon 2010). p53 gene transcribes multiple splice variants—one among them is an isoform g. There are many cellular tumour antigen p53 isoforms; they include p53 isoform 'a', 'b', 'c','d', 'f', 'g', 'h', 'i', 'j' and 'k' (See Lander et al., Nature 409 (6822), 860-921 (2001)). Human p53 gene can express nine different forms of the p53 protein; these are p53β, p53γ, Δ 133p53, Δ133p53 β, Δ 133p53 γ, Δ 40p53, Δ 40p53 β, and Δ 40p53 γ (Khoury and Bourdon 2010). In this paper, we concentrate our analysis on CDS 'TP53' cellular tumour antigen isoform 'g'. From our inquiry, we have gleaned insights by which we been able to derive a 15 amino acid alanine-rich polyproline oligopeptide PAPAAPT*PAAPA*PAP from a 45 bp nucleotide sequence derived from p53 promoter sequence which reads as CCTGCACCAGCAGCTCCTACAC-CGGCGGCCCCTGCACCAGCCCCC. This sequence has a molecular weight of 27.72kDa and the sequence matches with APPVAPAPAAPTPAAPAPAPSWPLS sequence 75-89 isolated by custom immune-affinity chromatography (See Dehart et al. 2013, LC-MS data of this peptide). Following its translation, we are able to derive an oligopeptide containing equivalent number of alanine (7) and proline (7) residues and having one interresidue–threonine. Below we illustrate the restriction figestion map of this unique peptide.

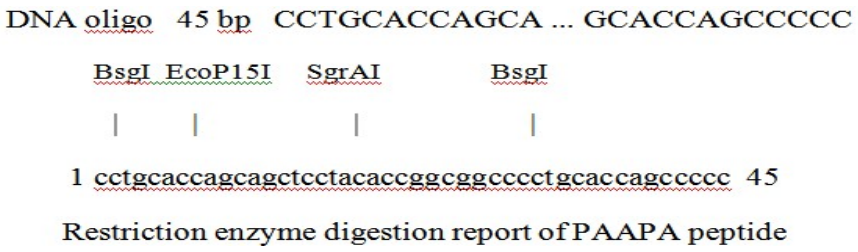

Figure 4. PAAPA peptide restriction digestion report (GenBeans 5.1)

To be noted, p53 gene products, along with its isoform 'g' are proline rich polypeptide chains. The total number of proline residues in this translated sequence amounts to 38 which accounts for 10.73% followed by serine residues which accounts for 9.04% of all the number of residues. We name this new proline-rich Oligo sequence as PAAPA (P_7TA_7) peptide since we tag this translated sequence containing an STS designated read PAAPA. The chemical formula of this peptide sequence PAPAAPT*PAAPA*PAP is C60H93N15O17 with a molecular weight of 1298.47 Da. Its theoretical pI is 5.95 and the peptide has an aliphatic index of 46.47. Design of proline-based cyclic oligopeptides has been attempted by Malešević et al., (2012). On searching proteomics chemical database Protein Resource Information (PRI) Uniprot, there was no standalone exact match of the fragment PAAPA (P_7TA_7) from the parent peptide, but we find this pattern existing as a component of Human Transmembrane protein 21, among others, which is a 385 aa protein and we find this sequence occurring at PGLGVPAAPAGAPEA 91-95. However, this protein is instable because its instability index (II) is computed to be high. The structure of the peptide is drawn below:

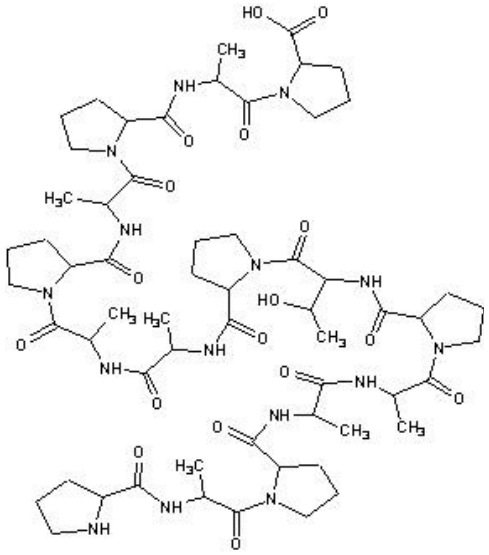

Figure 5. PAAPA P_7TA_7 peptide structure

As is evident, this structure contains several pyrrolidine ring structures because of the preponderance of cyclic protein residue proline. Proline is a ribosome substrate. Due to its unique structure, peptide chains containing several proline residues impede formation of peptide bonds by ribosomes which trigger ribosomal stalling (Melnikov et al., 2016) during translation elongation (See Joazeiro 2017 for ribosomal stalling). The significance of ribosomal stalling is yet undetermined in cancer biology, but the fact that cancer cells require the ribosomal machinery in order to proliferate and grow incessantly indicates that targeting ribosomal machinery, biogenesis or ribosome stalling in cancer cells may be a viable option to treat cancer. Using sequence from human p53 promoter region, we designed a specific primer with the following nucleotide sequence: TCCCATGTGCTCAAGACT having a 6aa translated product 'SHVLKT'. This peptide found its match in human OTU domain-containing protein 6A having a length of 288 aa. The peptide sequence matched with several other protein sequences including a protein named Nanos homolog1 from Brachydanio rerio (Zebrafish) whereas it matched with the following sequence ELRALSHVLKTPIEVI at position 226-231 of the human OTU domain chain on searching Protein Information Resource, an Uniprot protein search consortium. On oligomer analysis, we derived chemical properties of the aforementioned primer sequence having a GC content of 50%, Tm = 52.0°C [PCR], with MW 11.03 kDa. Data from peptide analysis shows that it has a MW = 683.8 and theoretical pI = 8.49. It has a relatively higher aliphatic index of 113.33. Following these, we fuse the two previous peptides designed so far—HRGRES and SHVLKT with the one PAAPA (P_7TA_7) peptide as an interresidual domain to derive a novel 27 aa protein structure given by 'HRGRESPAPAAPT*PAAPA*PAPSHVLKT'. Peptide analysis data shows that this protein has a MW of 2685.0 and pI of 10.84, but it is highly instable. Chemical modifications can improve stability of peptide drugs (Thundimadathil and Gangakhedkar (2014). Following single amino acid and dipeptide shuffling and sampling, several new configurations could be obtained from the given sample to obtain a more stable peptide. This could be attempted along with chemical modifications to obtain the most stable version of this polypeptide. This protein

is encoded by 83 nucleotides and its nucleotide analysis data yields a 69 bp ORF (ORF min. search Length trimmed to 25 bp) having a GC content of 66.66% with a MW of 21.03 Da. Within the ORF, several repeat units have been found, but two repeat units seem to be significant, each having 13 bp with their amino acid translation given as PLHQ which has been traced to more than several hundred matches on Uniprot Protein Information Resource Database.

3.2 Statistical Genomic Analysis Results

We have performed statistical analysis on all the eight exons so far derived from the p53 transcript variant 2 mRNA. The mean GC% content was found to be 56.19 with a standard deviation of 4.496. GC% contents also vary among the respective exons with the longest exon 8 having the lowest GC% content of 48.95%. Melting temperature Tm °Chas been found to vary as well in exons irrespective of their lengths with exon 3 having the highest Tm 86.65°C and lowest for exon 5 with Tm 79.99°C respectively .

4 Discussion

Cancer is "an abnormal process driven by mechanisms by which normal cells proliferate abnormally with compromised differentiation and metastasis" resulting due to mutations in tumour suppressor genes and abnormalities in DNA repair mechanism with resulting loss of control of normal cell cycle dynamics. It is due to deregulation and alteration of cell cycle dynamics which results in cancer and tumorigenesis. So cancer is often considered as the disease of the cell cycle (Malumbres and Barbacid (2001).). The driving forces of tumorigenesis are, however, the molecular mechanisms resulting from overexpression of oncogenes and loss of tumour suppressor genes (Guo et al., 2014). It is for these reasons that current therapeutic modules are being targeted after oncogenes and tumour suppressor genes. Nevertheless, cancer needs several critical questions to be answered in finer detail; for instance, what accounts for absence of growth regulation in cancer cells? What differentiates between stem cells being functional and being cancerous? What factors determine how tumour suppressor genes like p53 decides DNA repair and cell survival or apoptosis of the damaged cells? What abrogates the suppressive ability of p53 in suppressing tumourigenesis (Baker et. al., 1993)? What methods could be developed to restore endogenous normal p53 functions? How cancer cells avoid and escape apoptosis? What novel therapeutic modalities and drugs needs to be designed that targets existing and evolving pathways in cell cycle dynamics that augments apoptosis of cancer cells? How novel approaches like peptide-based therapeutic agents could be used in the treatment of human cancers? How to design more stable peptide compounds based on the understanding of what makes a protein stable (or unstable)? What novel peptidomimetic drugs could be designed for selective inhibition of malignant cells? These are among several important questions that call for answers in greater detail. And vigorous global efforts have sought for permanent answers to these questions resulting in great strides in cancer chemotherapy in recent years. But cancer is still one of the leading causes of death worldwide. There-

fore, investigators are seeking for potential therapeutics beyond the conventional chemotherapy regimens in order to discover and design novel agents based on the ability of peptides to affect cancer cells and cancer growth dynamics differently. Peptide-based cancer therapeutics is attracting increased attention and peptides are being increasingly seen as potential therapeutic agents in cancer nanomedicine (Zhang, Eden and Chen 2012). Several different kinds of peptides have already found their use in cancer therapeutics (See Aina et. al. 2002, Thundimadathil 2012, Xiao et al. 2015). For instance, great strides have been made in monoclonal antibodies-based treatment of hematologic malignancies (Reff et. al. 2002) and vascular targeting agents (Thorpe 2004). In small antibody mimetics, peptides finding applications in cancer nanomedicine are used as molecular targeting agents such as drug carriers, targeting ligands, and protease substrates (Zhang, Eden and Chen 2012), as well as peptide-based receptor blockers, hormonal agents, and tumour-homing peptides (Enb¨ack and Laakkonen 2007)

On this regard, we approach in a multistep mode to explore novel ways and methods to design putative peptide-based compounds which could be of interest for further analysis of and development of new lead molecules. The goal of this paper is to design and develop novel putative therapeutic peptides or compounds of interest which could be further developed into lead candidates for drug development aimed for treatment of cancer that aims specific processes in the genesis of cancer; i.e., suppression of oncogenes or reactivation of tumour suppressor genes. There exist many peptides that are used in the diagnosis and treatment of cancer (See Xiao et al., 2015). Peptide-based vaccines are being developed in clinical oncology for treatment against pancreatic cancer (Nishida et al 2014), gastric cancer (Masuzawa et al.), colorectal cancer (Seraino et al.), breast cancer (Mittendorf et al.), lung cancer (Rayaprolu et al.) and prostate cancer (Noguchi et al.). For a more descriptive account and a complete review of peptide-based treatment of cancer, see Xiao et al. (2015). Our research targets p53 exons and their protein translations, among others, its DNA-binding domain where most mutations have been found to occur. Also, we designed exon-specific primers to generate oligopeptides that might have therapeutic value by their modification, structural alteration and by addition of other functional groups fused into the designed oligopeptide. It is important to note that p53 is a potent tumour suppressive agent and whose cellular dynamics is correlated with suppression or genesis of cancer. In cancer cells, accumulation of p53 due to DNA damage and lack of expression of Apaf-1 indicates that malignant cells escape apoptosis. Our primary aim is to develop novel putative therapeutic peptides that may act within the cell to augment and enhance the levels of p53 which appears to be suppressed in cancers (Vousden and Lane 2007). We perform modelling of such compounds based on data obtained from chemical structural analysis of oligopeptides designed by novel mechanisms.

The key points regarding p53 (Refer to Vousden and Lane 2007) are as follows:

1. p53 is an essential tumour suppressor protein which becomes dysfunctional in cancers

2. This protein becomes mutant due to mutations in its promoter region

3. p53 activates cell cycle arrest in response to stress and DNA damage and makes damaged cells to undergo repair or apoptosis.

4. All these functions proves that it suppresses tumorigenesis and prevents DNA damage and development of cancers

5. p53 responds to genotoxic stress, DNA damage (double strand breakage) and activation of oncogenes

6. p53 accumulates in cancer cells

7. Too much overexpression of p53 may lead to early aging and neurodegenerative diseases. It is also responsible for senescence.

8. Its cellular levels are under control of HDM2/MDM2 which induces is proteasomal degradation

9. That it is a potential therapeutic target in the treatment of cancers.

This endogenous cellular protein, p53— is a potent tumour suppressor agent whose levels rise in response to DNA damage, is under negative feedback control of HDM2 (also known as MDM2). Activation of p53 involves three different steps: p53 stabilization, DNA binding, and transcriptional activation (Kruse and Gu 2009). Several attempts are in way to design novel tumour suppressor agents similar to p53, or stimulate mechanisms that results in induction of p53-dependent transcriptional activation of cell cycle arrest and activation of apoptotic genes. We perform rigorous genomic and proteomic analysis of p53 gene and its product—p53 protein, and report some distinctive features which could be exploited in designing therapeutic leads for treatment of cancer. We attempt to design a bioactive peptide compound that contains an oligopeptide derived from the HDM2-p53 fusion complex.

The cells in our body are under constant attack from stress and environmental genotoxins which often leads to DNA damage. Errors in DNA repair mechanism can lead to mutations that can alter the functions of tumour suppressor genes and hence, may lead to development of cancer (William and Schumacher 2016). There are various classes of tumour suppressor agents working in harmony to oversee cellular and DNA damage in response to genotoxins. The function of tumour suppressor gene or antioncogene is to guard the cells from becoming malignant; it protects the cells from becoming cancerous. p53 plays a central role in response to DNA damage by modulating and moderating a variety of DNA-damage response mechanisms (Vogelstein, Lane, and Levine 2000). The gene that encodes p53 tumour suppressor protein is TP53 gene. Loss of and mutation in this gene are involved in the pathogenesis of a variety of cancers which include leukemias, lymphomas and sarcomas. Other tumour suppressor agents include DNA repair proteins, and repressor genes that are essential for inhibiting cell division. p53 mediates cell cycle arrest and transcriptional activation of apoptotic genes. These include p21, PUMA, and BAX genes (Chipuk et al., 2004). The levels of p53 are regulated by several mechanisms inside the cell; i.e., p53 is kept in check by HDM2/MDM2. Regulation of p53 gene is also controlled by methylation, acetylation, phosphorylation and degradation. Methylation of Cytosine (C) and deacetylation of histones generally silences a gene (Baylin 2005, Soengas 2001, Jones 2001). For instance, CpG islands in a gene which are rich in GC% content are targets for methylation. Schroeder and Mass (1997) state that

the transcriptional activity of the promoter region of human p53 tumour suppressor gene is inactivated due to CpG methylation. Their work investigated the effect of CpG methylation on the functioning of transcriptional start sites within a specific region of the p53 promoter. Also, Pogribny et.al., (2000) previously showed that the expression of p53 gene is reduced due to single site mutation within the promoter region of this gene. In fact, addition of methyl groups (CH3) to cytosine nucleotides (but not guanine) results in epigenetic gene silencing (Soengas et al., 2001), for the reason that they are high GC methyl-acceptor regions of a sequence. Such gene silencing by cancer cells inactivate cancer related genes which in turn inactivate the apoptosis pathway. Therefore, DNA methylation inhibitors have the potentiality to reactivate cancer suppressor genes (Lübbert, 2000, Gros et al., 2012) and are viable candidates in the treatment of cancers. In essence, methylation is essential in controlling gene expression under normal circumstances. Seen both ways, silencing of tumour suppressor genes like p53 and DNA repair protein MLH1 among others predisposes cells towards mutagenesis, DNA damage and cancer genesis, while on the other hand, DNA methylation can protect us from cancers due to suppressing of tumour inducing oncogenes. For, DNA damage can be induced by oncogenes which could lead to development of cancers (Halazonetis, Gorgoulis, & Bartek, 2008).

HDM2 (also known as MDM2) is one of the negative regulators of p53 which is an ubiquitin E3 ligase that binds to p53 and inactivates it. MDM2 is a cellular antagonist of p53 that maintains and keeps balances of its cellular levels (Chi et al., 2005). HDM2/MDM2 however, is under control of $p14^{ARF}$ protein which confines MDM2 to nucleolus (Soengas et al., 2001). Therefore, it is $p14^{ARF}$ that prevents p53 from breaking down. In fact, it is another protein known as Death-associated protein kinase (DAPK) which transmits stress signals to $p14^{ARF}$. Down-regulation or loss of $p14^{ARF}$ due to mutation or abnormal methylation of the promoter of $p14^{ARF}$ leads to disruption in apoptotic signalling pathway due to the fact that the levels of HDM2/MDM2, which are under control of $p14^{ARF}$, rises up and induces proteolysis of p53. Therefore, the levels of p53 are kept in check by a pathway that involves DAPK, $p14^{ARF}$, and HDM2/MDM2 (Soengas et al., 2001). The mechanism by which p53 promotes cell suicide (apoptosis) involves several other signal peptides, proteins or enzymes which include BAX, Cytochrome C, *Apaf-1* and procaspase-9. P53 stimulates BAX which induces release of Cytochrome C that activates Apaf-1. This *Apaf-1* protein further downstream activates the enzyme procaspase-9 which leads to cell death/apoptosis. In malignant melanoma cells, *Apaf-1* is inactivated (Soengas 2001) so that the cancerous cells do not proceed to apoptosis but proliferate. But p53 mutation—although occurs aggressively in many different forms of cancers, is rarely observed in malignant melanoma. However, there still remains much to be known about the mechanisms and the factors that determine how p53 decides whether to repair *damaged* cells or allocate them for apoptosis; that is to say, what other factors are involved in p53 dependent cell survival or apoptosis? p53 activates DNA repair proteins at the G_1/S regulation checkpoint. In fact, p53 induces p^{21} (WAF_1) to bind to the G_1-S/CDK complex inhibiting their activity (Harper et al., 1995, Bartek and Lukas 2001). The protein p^{21} acts as a "stop" signal for cell division in the G_1 phase. Since abolition of S-phase entry leads to differentiation, it is important to identify specific molecules that play essential roles G_1/S transition in the cell cycle.

Malignant transformation of normal cells results on account of the combined effect of inactivation of tumour suppressors and activation of oncogenes. The p53 protein being a potent tumour suppressor, its role in human cancers have been well established based on researches that points to the fact that loss of function and mutations in TP53 genome leads to lower levels of this protein in response to DNA damage (Kruse and Gu 2009). Therefore, dysfunctional p53 leads to errors in cell cycle regulation. Since it is a potent tumour suppressor, the effects of mutagens modifying or damaging TP53 gene leads to abnormal p53 protein which fails to activate or repress genes responsible for downstream actions those related to DNA repair or apoptosis. Normally, the cell cycle is under tight control by a myriad of signal peptides which regulate the progression of cell growth and proliferation. DNA damage targets Cyclin-dependent kinases (CDKs) by p53-dependent induction of p21(Harper et al., 1995). Besides, a protein of interest is the retinoblastoma pRB tumour suppressor protein (Sage et al., 2000) whose active form prevents cells from crossing the G_1 checkpoint. This gene—RB (retinoblastoma gene) has been implicated in a wide variety of human cancers including familial retinoblastoma, osteosarcomas, and other forms of cancers (Goodrich and Lee 1993). The cyclin dependent complex CyclinD/Cdk4 phosphorylates, and inactivates pRb from its activated hypophosphorylated state (See Fig.6). In its hypophosphorylated form, activated RB binds to a subset of E2F complexes that turns them into suppressors which constrain expression of E2F target genes (Sherr 1996). Inactivation of Rb through phosphorylation leads to progression of the cell cycle past the G_1 checkpoint.

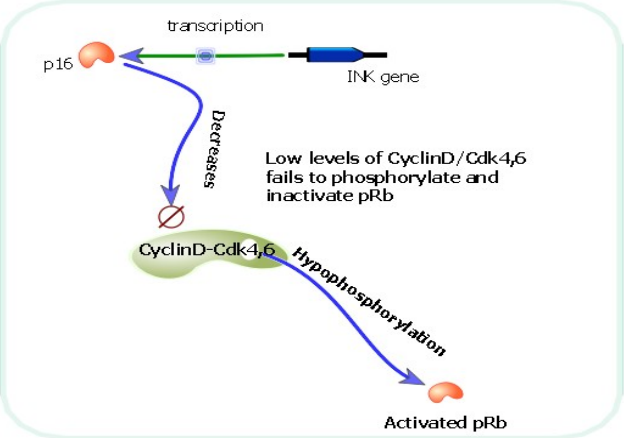

Figure 6. The retinoblastoma pRB activation-deactivation pathway.

On the other hand, in G_1 phase, p16 inactivates Cyclin D/Cdk4, 6 complex which fails to inactivate Rb. Transcription of INK gene leads to expression of p16 which decreases the levels of CyclinD-Cdk4, 6 which leads to hypophosphorylation of Rb. The three putative bioactive peptides that we have designed so far, HRGRES, SHVLKT from p53 exons and the PAPAAPT*PAAPA*PAP peptide from the promoter region sequence of p53 gene might have some pharmacological actions since these peptide fragments appear within the functional domains of several proteins mentioned above which appears in the Protein Information Resource of the Uniprot database. Further analyses of these putative compounds are called for to understand their biological functions which we attempt in our following research.

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Conflict of Interest:

The author confirms that there is no conflict of interest.

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