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Insilico Analysis of TP53 proteins using phylogenetic approach

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Abstract: The p53 gene (TP53) is a gene that is mutated in many cancers, and is the most common gene mutation found in cancer cells. The gene is a type of tumor suppressor gene that codes for a protein that inhibits the development and growth of tumors. It has been coined "the guardian of the genome[20]," when inactivated, it can also play a role in the persistence, growth, and spread of a cancer that develops. In this paper P53 proteins are computationally analysed using phylogenetic analysis approach[2]. Evolutionary studies are very important in the field of biological research as it provides the basis for comparative genomics. The sequence of TP53 protein in human is downloaded from NCBI and compared with protein sequences of different organisms. Maximum likelihood approach is used for phylogenetic analysis among different organisms. This study will ultimately decipher the treatment of cancer in different organism and also will be useful in near future for further pathway analysis using different new research strategies[3].

Keywords

Tumor, Apoptosis, phylogeny, sequence alignment, cancer.

Introduction

The name **p53** was given in 1979 describing the apparent molecular mass; SDS-PAGE analysis indicates that it is a 53-Kilodalton (kDa) protein. In humans, the *TP53* gene is located on the short arm of chromosome 17. The gene spans 20 kb, with a non-coding exon 1 and a very long first intron of 10 kb. The coding sequence contains five regions showing a high degree of conservation in vertebrates, predominantly in exons 2, 5, 6, 7 and 8, but the sequences found in invertebrates show only distant resemblance to mammalian TP53.

P53 is a nuclear transcription factor with a pro-apoptotic **function**. Since over 50% of human **cancers** carry loss of **function** mutations in **p53** gene, **p53** has been considered to be one of the classical type tumor suppressors. Tumor suppressor genes code for proteins that function to repair damaged DNA so a cell can't become a cancer cell, or result in the programmed cell death or apoptosis of cells that can't be repaired. They may also have other functions important in cancer growth, such as playing a role in regulating cell division or angiogenesis (the growth of new blood vessels to feed a tumor).

A mutation in the p53 gene (located on chromosome 17) is the most common mutation found in cancer cells and is present in over 50% of cancers. Talking about gene mutations and cancer, especially with tumor suppressor genes is confusing, because there are two primary types: germline and somatic.

Germline mutations (heritable mutations) are the type of mutations people may be concerned with when wondering if they have a genetic predisposition to cancer. The mutations are present from birth and affect every cell in the body. Genetic tests are now available to check for several germline mutations that increase cancer risk, such as mutated BRCA genes. Germline mutations in the TP53 gene are uncommon, and associated with a specific cancer syndrome known as Li-Fraumeni syndrome[20].

People with Li-Fraumeni syndrome often develop cancer as children or young adults, and the germline mutation is associated with a high lifetime risk of cancers such as breast cancer[8], bone cancer, muscle cancer, and more.

Somatic mutations (acquired mutations) are not present from birth but arise in the process of a cell becoming a cancer cell. They are only present in the type of cell associated with the cancer (such as lung cancer cells), and not other cells in the body. Somatic or acquired mutations are by far the most common types of mutations associated with cancer.

The p53 gene may be damaged (mutated) by cancer-causing substances in the environment (carcinogens) such as tobacco smoke, ultraviolet light, and the chemical aristolochic acid (with bladder cancer). Often times, however, the toxin leading to the mutation is unknown. If the gene is inactivated, it no longer codes for the proteins that lead to the functions noted above. Thus, when another form of DNA damage occurs in another region of the genome, the damage is not repaired and may result in the development of cancer.

There are a wide range of cancers that are associated with mutations in the p53 gene. some of these include: Bladder cancer, Breast cancer: The TP53 gene is mutated in around 20 percent to 40% of breast cancers, Brain cancer (several types), Cholangiocarcinoma, Head and neck squamous cell cancer, Liver cancer, Lung cancer, Colorectal cancer, Osteosarcoma (bone cancer) and myosarcoma (muscle cancer), Ovarian cancer, Adrenocorticol carcinoma.

Some studies include bayesian phylogenetic methods and a Markov chain Monte Carlo algorithm which are used to obtain the most probable trees and posterior probabilities of clades [18].

One study found that p53 and Myc proteins were key to the survival of Chronic Myeloid Leukaemia (CML) cells[19]. Targeting p53 and Myc proteins with drugs gave positive results on mice with CML.

In one study it was seen that body size and metabolic rate or body size and age at maturation, body size and lifespan are strongly correlated such that larger species tend to live longer[7] than smaller species as in some organisms TP53 [1] copy number increases which ultimately saves it from cancer like in steppe mammoth, Minke Whale and Human[14].

P53 has become current focus for cancer research[16]. Lot of research work is going on the function and control of P53 and hope to develop medicines[6] for the cure of cancer. Phylogenetics is the basis for comparative genomics[4] and helps in analysis of complete family history of different organisms and thus deriving genetic variation[13] and relationships among them.

Finding all the functional parts of DNA or protein sequences and using this information to improve the health of individuals and society are the focus of the next phase of the Human Genome Project[17]. Comparative analyses of genome sequences will be a major part of this effort[5].

Methodology:

The sequence of Homo sapiens tumor protein p53 (TP53) was retrieved from the NCBI (http://www.ncbi.nlm.nih.gov) in FASTA format. Also Cellular tumor antigen P53 Sequence of other organisms were retrieved from NCBI[15].

Now MEGA software version 11.01 was downloaded[10].

After retrieval of sequences these sequences are aligned. So Multiple sequence alignment is performed using MEGA Software Muscle Alignment package[9].

Next step was to perform Phylogenetic analysis of H. sapiens tumor protein p53 (TP53) and all other organisms using MEGA software[11,12]. Phylogenetic tree was constructed by the software showing the ancestral relationship among the sequences using Maximum Likelihood method of phylogenetic analysis and Bootstrap method of testing. It took more than one hour. The tree forms different clusters showing their relationship with each other. The sequences which lie in the same cluster are closely related. The tree calculated different distance matrices.

Maximum likelihood estimation involves defining a likelihood function for calculating the conditional probability of observing the data sample given a probability distribution and distribution parameters. This approach can be used to search a space of possible distributions and parameters.

Results and Discussion

The sequences of Homo Sapiens TP53 and other organisms were retrieved from NCBI database. The organisms are Sus Surcoma (pig), Pan Troglodytes (Chimpanzee), Felis Catus(domestic Cat), Bos Taurus(Cattle), Ovis Aries(Sheep), Sus Scrofa(wild pig), Cavia Porcellus(Domestic guinea pig), Mesocricetus Auratus(Gloden Hamster), Delphinapterus Leucas(Beluga Whale) and Oryctolagus Cuniculus(Rabbit). Then these sequences are aligned using Muscle alignment mode of MEGA Software.

Fig 1 Alignment of sequences

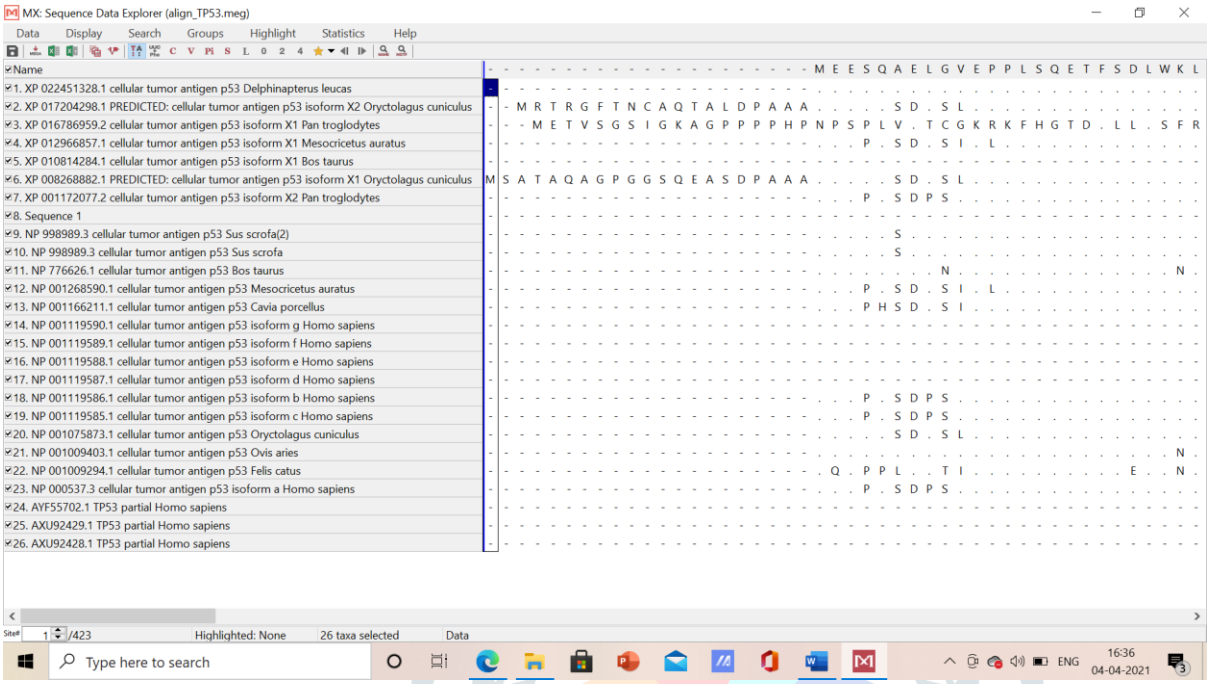
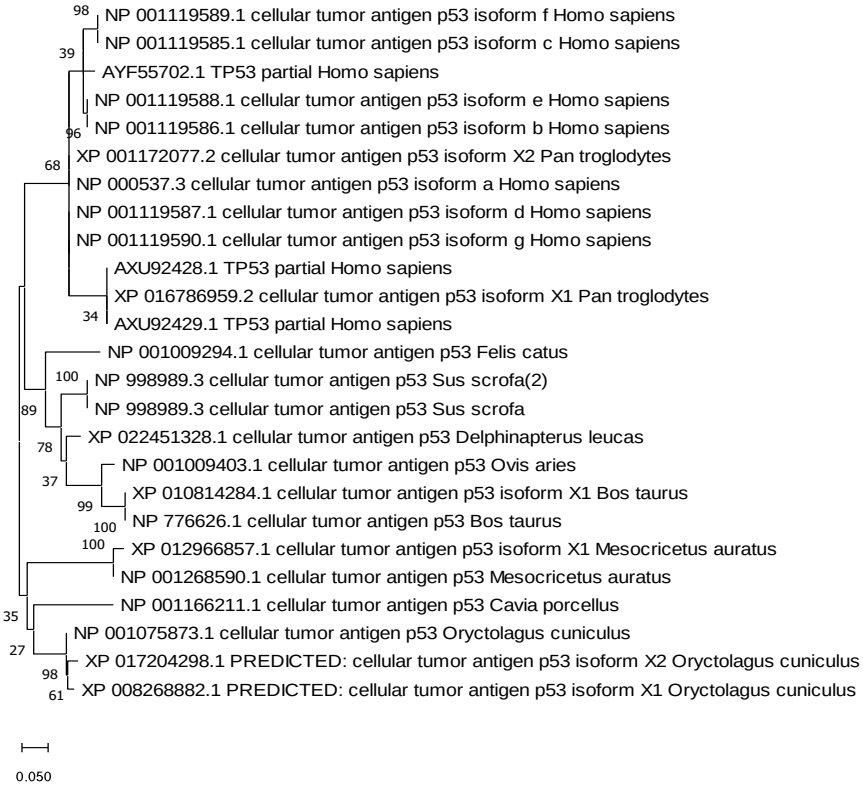
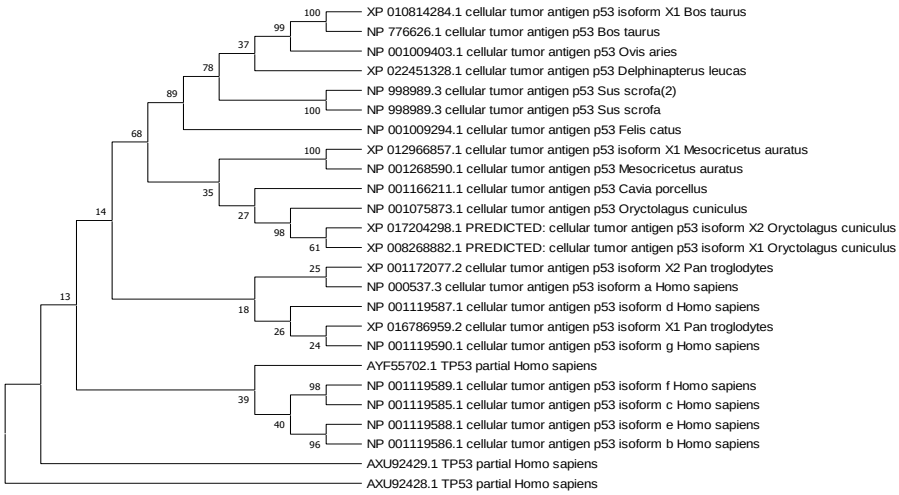


Fig2 Phylogenetic Analysis Using MEGA software : Original tree Fig3 Bootstrap tree





Evolution of the TP53 was studied in different organisms and adaptive changes were in the sequences. Organism that originated from same ancestors are placed in same clusters whereas those which are distant from each other are placed in separate clusters. Majority of human p53 sequences are lying in the same clusters. P53 protein of Bos Taurus(cattle) are distantly related with the H. sapiens tumor protein p53 but closely related to Ovis Aries(Sheep). Felis Catus is closely related to Ovis Aries(sheep) but distantly related to human TP53.

One of the study says as time passes, healthy cells are more likely to become cancerous because more and more damaging mutations accumulate in the cell's DNA. Assuming that all cells have a similar risk of acquiring mutations, larger and longer-

lived animals – like elephants – should have a higher risk of cancer than smaller, shorter-lived animals – like mice. However, there does not appear to be any link between the size of an animal and its risk of developing cancer. Consequently, a key question in cancer biology is how very large animals protect themselves against these diseases. Body size and metabolic rate or body size and age at maturation, body size and lifespan are strongly correlated such that larger species tend to live longer than smaller species. In this paper study says the same through phylogenetic analysis and relation between Delphinapterus leucas (Beluga whale) and human is seen. The various organisms studied here can also be related by size and it can be said that risk of cancer is less in Whale and human compared to rabbit as the TP53 copy will increase in these organisms, if this is not the case rabbit will be at low risk compare to whale.

Conclusion

This work can be further taken to next level and can go for docking.

References

1) Abegglen LM, Caulin AF, Chan A, Lee K, Robinson R, Campbell MS, Kiso WK, Schmitt DL, Waddell PJ, Bhaskara S, Jensen ST, Maley CC, Schiffman JD, Potential mechanisms for cancer resistance in elephants and comparative cellular response to DNA damage in humans. JAMA 314:1850–1860. doi: 10.1001/jama.2015. 13134, PMID: 26447779
2) Brooks DR, Jaret B, Charmaine C, David CE, Kaila EF, Jörg F, Dominik H, Stephanie H, Deborah AM, Michelle M, Linda AT, Jessica LW, Niklas W, David Z, David Z (2007). Quantitative Phylogenetic Analysis in the 21st Century. Revista Mexicana de Biodiversidad. 78: 225-252.
3) Ford MJ , Effects of natural selection on patterns of DNA sequence variation at the transferrin, somatolactin, and p53 genes within and among chinook salmon (Oncorhynchus tshawytscha) populations. Mol. Ecol. 9: 843-855, 2000.
4) Soltis DE, Soltis PS, The Role of Phylogenetics in Comparative Genetics. Plant Physiol. 132: 1790-1800, 2003.
5) Hupp TR, Lane DP, Ball KL , Strategies for manipulating the p53 pathway in the treatment of human cancer. Biochem. J. 352(1): 1-17, 2000.
6) Lane DP, Hupp TR , Drug discovery and p53. Drug Discov. Today, 8: 347-355, 2003.
7) Campisi J., Cancer and ageing: rival demons? Nature Reviews Cancer 3:339–349. doi: 10.1038/nrc1073, PMID: 12724732, 2003.
8) Donehower LA, Does p53 affect organismal aging? Journal of Cellular Physiology 192:23–33. doi: 10. 1002/jcp.10104, PMID: 12115733, 2002.
9) Edgar RC., MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32:1792–1797. doi: 10.1093/nar/gkh340, 2004
10) Sudhir Kumar, Masatoshi Nei, MEGA: A biologist centric software for evolutionary analysis of DNA and protei sequences, Brief Bioinformatics, 2008.
11) Kumar S, Tamura K, Jakobsen IB, et al. MEGA2: molecular evolutionary genetics analysis software. *Bioinformatics.* 2001; 17:1244–1245.
12) Tamura K, Dudley J, Nei M, et al. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol Biol Evol.* 2007; 24:1596–1599.
13) Mohammad Haroon Khan, Hamid Rashid and Asif Mir, Phylogenetic Analysis of human TP53 gene using computational approach, African Journal of Biotechnology Vol. 10(3), 2011.
14) Sulak et al., TP53 copy number expansion is associated with the evolution of increased body size and an enhanced DNA damage response in elephants, vol5 ,11994, eLife 2016.
15) National Centre for Biotechnology Information, Bethesda, National library of Medicine, U.S[1988], cited 2017 April6. Available: https://www.ncbi.nlm.nih.gov/
16) Jingjie et al., SIRT1 and P53, effect on cancer, senescence and beyond, Biochim Biophys Acta 2010.
17) Collins et al., A vision for the future of genomics research , Nature 422, 835-847, 2003.
18) Hudelot et al, RNA based phylogenetics methods: application to mammalian mitochondrial RNA sequence, Molecular phylogenetics evolution, vol 2, 241-52, 2003.
19) Abraham et al., Dual targeting of P53 andc-Myc selectively eliminates leukaemic stem cells, Nature, 534,341-346, 2016.
20) Mantovani et al., Mutant P53 as a guardian of the cancer cell, Cell death and Differentiation, 26,199-212, 2019.