

Phylogenetic analysis of Microtubulin

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Abstract-Tubulin is a polypeptide which help in the emergence of microtubule, and it is the major element for the anatomical structure of microtubules. Microtubules are the medial fiber and constituents of the cytoskeleton through which shape of the nucleus is controlled, they complex for different tasks like in the group of mitotic apparatus throughout cell division, cell growth or in the axon extension of neuronal units.

Historically the main part of microtubule having many subunits such as alpha and beta tubulin (heterodimers) are associated in a head-to-tail fashion giving rise to microtubule polarity. Gamma tubulin is found at microtubule-organizing centers such as the spindle poles or the centrosome suggesting that it is involved in minus-end cloud seeding of microtubule assembly .(11)Delta and epsilon tubulin are mostly related to alpha and beta tubulin ,in most of the species they help in regulation of centriole function. (12)Zeta tubulin is the last member of tubulin ancestry and mostly un characterized and absent in humans . In this paper we performed phylogenetic analysis of tubulin protein in various organisms such as human, chimpanzee, octopus, rice plant, rat, tortoise, archaeon, saccharomyces, frog, zebra finch using multiple sequence alignment tools mega software molecular evolutionary genetics analysis by identifying the relations we can further try editing the gene from the functional one after finding the close proximity create vaccines or anticancer drugs

INTRODUCTION

(10)Phylogenetics is the study of developmental relationships among biological systems often species or genes which may be referred to as taxa. A phylogenetic tree is known as phylogeny which is a diagram that displays the developmental relation of various organisms or genes from common ancestor.

Phylogeny is useful for arranging knowledge of the ancestor of biological diversity for construction and classification. (6)

Tubulin is a protein, containing subunits alpha, beta, gamma, delta, epsilon, zeta among all of them alpha, beta and gamma tubulins are most important and polymerize during cell division to form microtubules (7). Alpha and Beta (heterodimer) tubulins are useful for anticancer drugs like vinblastin vincristine and paclitaxel. Tubulin exists in various isoforms(1) which are expressed by specific genes some of these isotopes are conveyed differently in standard and neoplastic nucleus. Microtubules are helical arrangements of alternating spherical units of alpha-tubulin and beta-tubulin having molecular mass of 50000dal with homogeneous amino acid configuration (2) They form compositions of rigid vacant fibrous shafts of spherical protein tubulin measuring around 25 nm in diameter the and tubulins are encoded by small families of related genetic code that combine to form a dimers in individual.

Beta-tubulin is of particular interest because it forms a type of microtubule that is indicated exclusively in nerve cells and for the fact that the drugs bind to tubulin making it as a study of interest as a therapeutic target in treating human cancers, on the other hand tubulin is known to be accountable for the nucleation of microfilament the macromolecules that are been identified in biogenesis.(13) Gamma tubulin ,this molecule present in centrosome which helps in microtubule nucleation.(14) Delta and Epsilon tubulin are require for the microtubule stability in centrioles and basal bodies, and located at non-centriolar structures.(12) Zeta tubulin are mostly un

characterized and absent in humans, in xenopus zeta tubulin is component of basal foot, centriolar appendage which connects centrioles to apical cytoskeleton.

(3). Mutations in tubulin gene lead to abnormal neural migration and organization, differentiation and axon guidance with motor impairment intellectual disability and (9) epilepsy. Mutations in tuba1a tubb2b and tubb3 genes are connected with epilepsy it has been found that pseudo genes exist for the beta-tubulin and sequencing errors results in erroneous interpretation determining true mutations in tubulin leading to brain malformations. (11) Mutations of delta and epsilon tubulin cause centriole destabilation and uncommon mitosis, even cell death in serious cases

MATERIALS AND METHODOLOGY

1. NCBI (National Center for Biotechnology Information)

<https://www.ncbi.nlm.nih.gov/>

NCBI has a multi-disciplinary research group composed of computer scientists, molecular biologists, mathematicians, biochemists, research physicians, and structural biologists concentrating on basic and applied research in computational molecular biology. These investigators not only make important contributions to basic science but also spring up with new methods for applied research activities. Together they are studying fundamental biomedical problems at the molecular level using mathematical and computational methods. These problems include gene organization, sequence analysis, and structure prediction. A sampling of current research projects includes: detection and analysis of gene organization, repeating sequence patterns, protein domains and structural elements, creation of a gene map of the human genome, mathematical modelling of the kinetics of HIV infection, analysis of effects of sequencing errors for database searching, development of new algorithms for database searching and multiple sequence alignment, construction of non-redundant sequence databases, mathematical models for estimation of statistical significance of sequence similarity, and vector models for text retrieval. Additionally, NCBI investigators maintain ongoing collaborations with several institutes within the NIH and also with numerous academic and government research laboratories.

ACCESSION NUMBERS: 1. 0805287A(Homo sapiens-Human), 2. NP_001038974.1 (Pan troglodytes-chimpanzee), 3.AWD75470.1 [Callistoctopus minor-octopus], 4.AAB84298.1 (Oryza sativa indica-rice), 5. BAA36504.1 (Rattus norvegicus-rat), 6.: XP_030399692.1 [Gopherus evgoodei- tortoise], 7.RYY38320.1(Archaeon-bacteria), 8. GHM91935.1 (Saccharomyces cervisiae-yeast), 9. AAA49977 (Xenopus laevis- Frog) 10. XP_030118315 [Taeniopygia guttata- zebra finch]

2. EBI European Bioinformatics Institute:

<https://www.ebi.ac.uk/>

EMBL-EBI hosts a vast amount of molecular data and other information that is indexed by EBI Search. This includes gene and protein sequences, protein families, structures, gene expression data, protein interactions, pathways and small molecules, to name a few. One can search across academic literature and patents as well as information about the institute and its works. In EBI Search boxes you can enter any meaningful term to find relevant information by typing. In EBI we use CLUSTAL OMEGA for Multiple sequence alignment (phylogenetic tree)

Multiple Sequence Alignment (MSA) is generally the alignment of three or more biological sequences (protein or nucleic acid) of similar length. From the output, homology can be inferred and the evolutionary relationships between the sequences studied. Most multiple sequence alignment programs use heuristic methods rather than global optimization because identifying the optimal alignment between more than a few sequences of moderate length is prohibitively computationally expensive. On the other hand, heuristic methods generally fail to give guarantees on the solution quality, with heuristic solutions shown to be often far below the optimal solution on benchmark instances.

Clustal Omega is a multiple sequence alignment program that uses seeded guide trees and HMM profile-profile techniques to generate alignments between three or more sequences. It produces biologically meaningful multiple sequence alignments of divergent sequences. Evolutionary relationships can be seen via viewing Cladograms or Phylograms. A fast and accurate multiple sequence alignment algorithm for DNA and protein sequences. This tool uses the Wu-Manber string-matching algorithm, to improve both the accuracy and speed of multiple

sequence alignment. It is a fast and robust alignment method, especially well-suited for the increasingly important task of aligning large numbers of sequences.

3.MEGA SOFTWARE

<https://megasoftware.net/>

The objective of the MEGA software has been to provide tools for exploring, discovering, and analyzing DNA and protein sequences from an evolutionary perspective. The first version was developed for the limited computational resources that were available on the average personal computer in early 1990s. MEGA1 made many methods of evolutionary analysis easily accessible to the scientific community for research and education. MEGA2 was designed to harness the exponentially greater computing power and a graphical interface of the late 1990's, fulfilling the fast-growing need for more extensive biological sequence analysis and exploration software

Tubulin is a heterodimeric protein made up of polypeptide chains called monomers (alpha,beta tubulins) differ in amino acid sequence that helps in polymerizing the long chains into microtubules an essential element in cytoskeleton ,it is the major component in eukaryotic cytoskeleton .we can collect plant tubulin in the interphase nucleus during cold treatment.

First FASTA sequences and Accession numbers of different organisms 1. (0805287A(Human), 2. NP_001038974.1(Pan troglodytes-chimpanzee), 3. AWD75470.1 [Callistoctopus minor-octopus], 4. AAB84298.1 (Oryza sativa indica-rice), 5. BAA36504.1 (Rattusnorvegicus-rat), 6: XP_030396967.1 [Gopherusevgoodei- tortoise]7. RYY38320.1(Archaeon), 8. GHM91935.1 (saccharomyces cervisiae), 9. JAV48716.1 (Agkistrodoncontrixcontrix) 10.NP_001093160.1 [Taeniopygiauttata-s]are collected from protein data base of NCBI.

METHODOLOGY

- Downloaded MEGA software, opened main interface Click on alignment, selected edit/build up alignment in The displayed tool bar selected a new alignment
- Click on protein from corresponding tool bar, a main screen appears on the display where many tabs of sequences appeared ,copied FASTA sequences from NCBI are pasted in the main screen near sequences and selected an option named W from the ribbon .
- Click on a tiny bar naming align appears on the screen, then click on okay to complete parameters after clicking on okay we got alignment results saved results in the media.
- Opened main interface of downloaded MEGA software selected phylogeneic analysis from ribbon a tool bar appears on the main screen selected construct /test maximum likelihood tree.
- Selected ,saved alignment document from media, a dialogue box of completing parameters appears click on okay, results are shown on the display(fig:4)

```
#
#
# Percent Identity Matrix - created by Clustal2.1
#
#
```

| | | | | | | | | | | |
|--------------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| 1: [Callistoctopus | 100.00 | 82.26 | 30.66 | 34.76 | 34.48 | 32.41 | 34.02 | 34.02 | 34.02 | 34.63 |
| 2: [Rattus | 82.26 | 100.00 | 30.43 | 35.84 | 34.25 | 31.26 | 33.56 | 33.56 | 33.56 | 34.17 |
| 3: [Oryza | 30.66 | 30.43 | 100.00 | 40.14 | 40.82 | 39.91 | 41.95 | 41.95 | 41.95 | 41.50 |
| 4: [Saccharomyces | 34.76 | 35.84 | 40.14 | 100.00 | 72.91 | 71.17 | 75.23 | 75.23 | 75.23 | 74.83 |
| 5: [archaeon] | 34.48 | 34.25 | 40.82 | 72.91 | 100.00 | 82.39 | 87.81 | 87.81 | 87.81 | 88.26 |
| 6: HUMAN | 32.41 | 31.26 | 39.91 | 71.17 | 82.39 | 100.00 | 93.92 | 93.92 | 93.92 | 90.77 |
| 7: [Pan | 34.02 | 33.56 | 41.95 | 75.23 | 87.81 | 93.92 | 100.00 | 100.00 | 100.00 | 96.62 |
| 8: [Gopherus | 34.02 | 33.56 | 41.95 | 75.23 | 87.81 | 93.92 | 100.00 | 100.00 | 100.00 | 96.62 |
| 9: [Taeniopygia | 34.02 | 33.56 | 41.95 | 75.23 | 87.81 | 93.92 | 100.00 | 100.00 | 100.00 | 96.62 |
| 10: [Xenopus | 34.63 | 34.17 | 41.50 | 74.83 | 88.26 | 90.77 | 96.62 | 96.62 | 96.62 | 100.00 |

Figure 1 these are percent identity matrix results of 10 sequences Which are collected from ncbi ((0805287A(Human) , 2. NP_001038974.1(Pan troglodytes-chimpanzee),

3. AWD75470.1 [Callistoctopus minor-octopus)
4. AAB84298.1 (Oryza sativa indica-rice),
5. BAA36504.1 (Rattusnorvegicus-rat) ,
6. : XP_030396967.1 [Gopherusevgoodei-tortoise] ,
7. RYY38320.1(Archaeon) ,
8. GHM91935.1 (Saccharomyces cerevisiae) ,
9. JAV48716.1 (Agkistrodoncontrixcontrix)
- 10 . NP_001093160.1 [Taeniopygiaguttata-

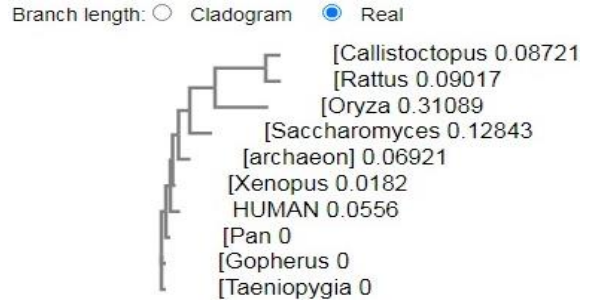


Figure- 2 this is the phylogenetic tree (clustal w from multiple sequence alignment EBI

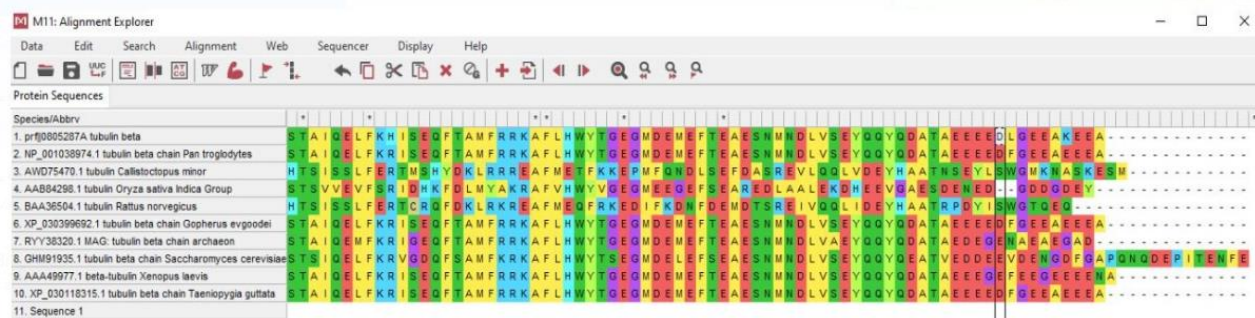


figure:3 these are alignment results ,from MEGA software

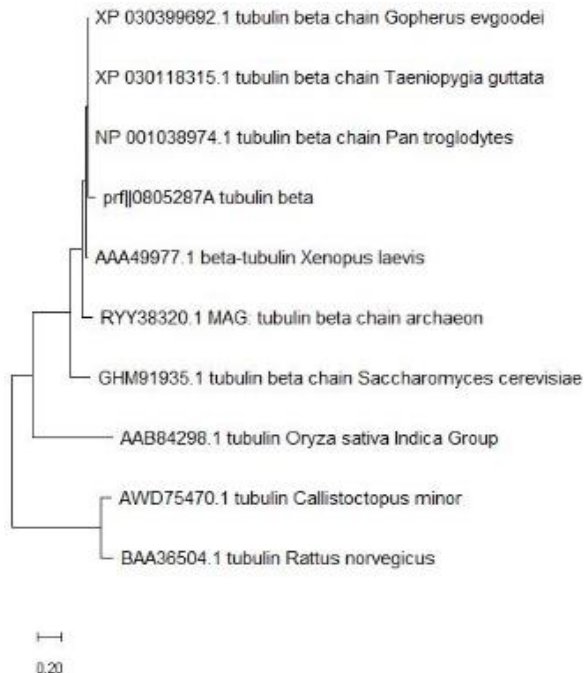


Figure:4 These are results of the phylogenetic analysis

CONCLUSION

Tubulin polypeptide are useful in the formation of microtubules and plays important role in nervous system in health and neurological diseases Parkinson's

and Alzheimer's set up for the research to support tubulin role in the development of new therapeutic drugs particularly for anticancer drugs hope this phylogenetic analysis performed by using protein sequence of different organisms belonging to different taxonomy phylogenetic results reveals that tortoise, zebra finch, chimpanzee and human are of one branch and closely related and evolutionary distance between chimpanzee , tortoise and zebra finch is zero this branch is next closely related to frog and this whole branch is related to archaeon and yeast, rice acts as a bridge between vertebrate and invertebrates and on the other side rat 0.09017and octopus 0.08721 are closely related with 1 branch ,clustal w phylogenetic tree and mega phylogenetic analysis are almost equal but clustal w gives results from low-high and mega software from high low current research is looking further into how new anticancer therapies can be developed by targeting tubulins and the microtubules that they create as it has been found that the dynamics of the mitotic spindle associated with mitotic arrest and cell death this is different to most other chemotherapeutic cancer drugs that act on dna at this point more research into tubulin and microtubules play a role in cancer and is required to access the full potential they pose to the development

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