International Journal of Zoology Studies
www.zoologyjournals.com
ISSN: 2455-7269
Received: 14-11-2023, Accepted: 29-11-2023, Published: 15-12-2023
Volume 8, Issue 4, 2023, Page No. 62-64

Study of *In Silico* analysis and phylogeny of some tumor antigens alphafetoprotein and mesothelin

Thakare P R[1], Yewale R M[1], Satpute M M[2], Gedam K R[3]
[1] Department of Zoology, Jijamata Mahavidylaya, Buldhana, Buldhana, Maharashtra, India
[2] Department of Zoology, Vivekanand Science College, Hiwra, Ashram, Buldhana, Maharashtra, India
[3] Research Scholar, Department of Zoology, Lokmanya Tilak Mahavidyalaya, Wani, Yawatmal, Maharashtra, India

Abstract
This article presents *In Silico* and phylogenetic tree analysis and role of tumor antigens alpha-fetoprotein and mesothelin. The 21st century saw a significant increase in knowledge about the fundamental role of alpha-fetoprotein in neoplastic processes. Alpha-fetoproetien is the biomarker of hepatocellular carcinoma whereas mesothelin a tumor associated antigen broadly overexpressed on various malignant malignant tumor cells. Statastical parameters *viz*., number of amino acids (in Daltons), number of acidic and basic amino acids, Radius of Folded Protein (A^0) Estimated pH for protein of alpha-fetoprotein and mesothelin were studied.

Keywords: *In-silico*, tumor antigens, hepatocellular carcinoma etc

Introduction

AFP is normally produced during foetal and neonatal development by the liver, yolk sac, and in small concentrations by the gastrointestinal tract. After birth, serum AFP concentrations decrease rapidly, and by the second year of life and there after only trace amounts are normally detected in serum. Alpha-fetoprotein is a protein that in humans is encoded by the *AFP* gene. The *AFP* gene is located on the q arm of chromosome 4.The AFP test is a test that is performed during pregnancy of maternal blood or foetal amniotic fluid at 16-19 weeks of gestation.

Mesothelin is a cell surface protein that is found in normal mesothelium and highly expressed in several cancers including mesotheliomas and ovarian and pancreatic cancers. Mesothelin is not a cancer-specific antigen, it is a differentiation antigen that is present on normal cells and highly expressed in many cancers. It is produced as a part of the 69 kDa precursor protein. The furin cleavage of the precursor protein yields two proteins, the N-terminal megakaryocyte potentiating factor (MPF), which is a soluble extra-cellular protein. Whereas MPF was isolated from the medium of a human pancreatic cancer cell line. Mesothelin might be involved in adhesion and particularly in adhesion and spread of ovarian cancer cells throughout the mesothelium lining of the peritoneal cavity.Phylogenetic & Evolutionary Biology is the field that deals with the study of evolutionary relations among groups of organisms and the computational simulation techniques for the study of biological, behavioral, and social systems. A phylogeny, or evolutionary tree, represents the evolutionary relationships among a set of organisms or groups of organisms, called taxa. The tips of the tree represent groups of descendent taxa (often species) and the nodes of the tree represent the common ancestors of those descendants.

Review of literature

Funda Çorapçıoğlu *et al., (*2004) studied on serum alpha fetoprotein levels in healthy full-term neonates and infants. Alpha fetoprotein (AFP) is an important tumour marker in childhood. However, AFP levels remain high during the first few months of life, making clinical interpretation difficult in this period. The aim of the present study is to determine normal AFP levels in healthy full-term neonates and infants followed-up at Kocaeli University Hospital, Department of Paediatrics.

Lisa H. Butterfield, James S. Economou, in Gene Therapy of Cancer (Second Edition), (2002) DNA and Dendritic Cell-Based Genetic Immunization against Cancer studied that Alpha-fetoprotein (AFP) is the most abundant serum protein before birth, and the levels decrease to very low but detectable levels after birth. AFP is reactivated by approximately 80% of HCC. Like PSA, levels of serum AFP are an important diagnostic tool for detection of HCC. Extensive epitope mapping has been performed which has identified four immunodominant and ten subdominant epitopes restricted by HLA-A2.1. They have found that T-cell responses to AFP can be generated in both murine *in vivo* and human *in vitro* systems. This work has shown that AFP peptides are processed and presented by the cellular machinery and that AFP antigen-specific effector T cells can be expanded.

M.E.C. Blohm *et al*., (1997) [1] studied the alpha,-fetoprotein (afp) reference values in infants up to 2 years of age.

Jingyu Zhan, Dong Lin, Nathan Watson, Wai Kwan Tang *et al*., (2023) [4] Structures of Cancer Antigen Mesothelin and Its Complexes with Therapeutic Antibodies studied that the tumor-associated antigen mesothelin is expressed at high levels on the cell surface of many human cancers, while its expression in normal tissues is limited. The binding of mesothelin to the tumor-associated cancer antigen 125 (CA-125) can lead to heterotypic cell adhesion and tumor metastasis within the pleural and peritoneal cavities. Immunotherapeutic strategies targeting mesothelin are being intensively investigated. Here, we report the crystal structures of mesothelin that reveal a compact, right-handed solenoid consisting of 24 short helices and connecting loops. These helices form a nine-layered spiral coil that resembles ARM/HEAT family proteins. Glycan attachments have been identified in the structure for all three predicted N-

glycosylation sites and confirmed with samples from cell culture and patient ascites. The structures of full-length mesothelin and its complex with the Fab of MORAb-009 reveal the interaction of the antibody with the complete epitope, which has not been reported previously. The N-terminal half of mesothelin is conformationally rigid, suitable for eliciting specific antibodies, whereas its C-terminal portion is more flexible. The structure of the C-terminal shedding-resistant fragment of mesothelin complexed with a mAb 15B6 displays an extended linear epitope and helps explain the protection afforded by the antibody for the shedding sites.

Christopher D. *et al*., (2006) studied the Real-time detection of mesothelin in pancreatic cancer cell line supernatant using an acoustic wave immunosensor.

Bangalore K Sathyanarayana1 *et al*., (2008) [6] studied and concluded that the mesothelin is a 40 kDa protein present on the surface of normal mesothelium cells and overexpressed in many human tumours, including mesothelioma and ovarian and pancreatic adenocarcinoma.

Material and methods

To analyse the protein Alpha-fetoprotein, and mesothelin the amino acid sequence of human Alpha-fetoprotein, and mesothelin was retrieved from NCBI site and was used for analysis in PepTool 2.0 demo version. For phylogenetic analysis, amino acid sequences of different vertebrate species ware retrieved from NCBI and were aligned using MEGA 7 program. The phylogenetic tree was constructed using the same aligned file and the tree was saved and analysed.

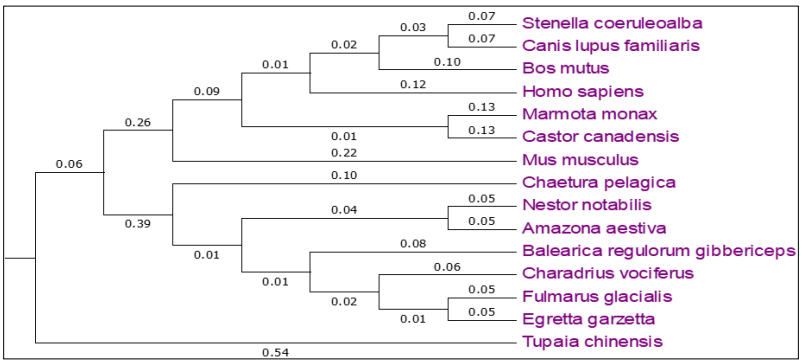
Observation and results

Protein Statistics

Alpha-fetoprotein

Sr. No	Statistical Parameter	Value	Explanation of Term
1	Molecular weight (Daltons)	55563.793	The sum total atomic weight for all the amino acids comprising the current sequence. Molecular weight calculations do not take into account post-translational modifications such as N-and C-terminal modifications or glycosylated residues
2	Number of amino acids	490	The total number of amino acids comprising the current sequences.
3	Number of basic amino acids	53	The sum total number of arginine (R) and lysine (K) residues comprising the current sequence. Basic amino acids carry a net positive charge at physiological pH (7.2).
4	Number of acidic amino acids	67	The sum total number of asparatic acid (D) and glutamic acid (E) residues comprising the current sequence. Acidic amino acids carry a net negtive charge at physiological pH (7.2).
5	Est. Radius of Folded Protein (A^0)	30.5495	The estimated radius, in Angstroms, for the current sequence, assuming it folds into a globular protein. The radius is defined as the cube root of the number of amino acids comprising the sequence multiplied by the average distance between adjacent amino acid C-alpha atoms (3.875 Angstroms).
6	Estimated pH for protein	6.2	The pH at which the protein carries a net zero charge.

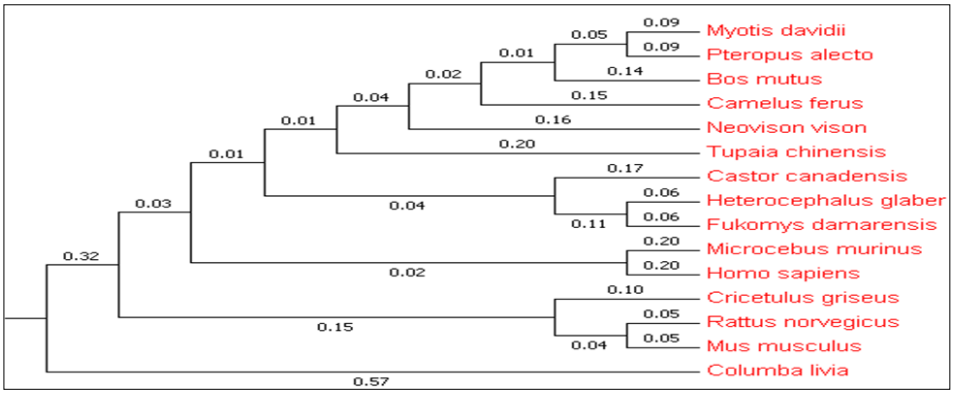
Phylogenetic tree of Alpha-fetoprotein



Mesothelin

Sr. No.	Statistical Parameter	Value	Explanation of Term
1	Molecular weight (Daltons)	68010.656	The sum total atomic weight for all the amino acids comprising the current sequence. Molecular weight calculations do not take into account post-translational modifications such as N-and C-terminal modifications or glycosylated residues
2	Number of amino acids	622	The total number of amino acids comprising the current sequences.
3	Number of basic amino acids	63	The sum total number of arginine (R) and lysine (K) residues comprising the current sequence. Basic amino acids carry a net positive charge at physiological pH (7.2).
4	Number of acidic amino acids	70	The sum total number of aspartic acid (D) and glutamic acid (E) residues comprising the current sequence. Acidic amino acids carry a net negative charge at physiological pH (7.2).
5	Est. Radius of Folded Protein (A^0)	33.0777	The estimated radius, in Angstroms, for the current sequence, assuming it folds into a globular protein. The radius is defined as the cube root of the number of amino acids comprising the sequence multiplied by the average distance between adjacent amino acid C-alpha atoms (3.875 Angstroms).
6	Estimated pl for protein	6.4	The pH at which the protein carries a net zero charge.

Phylogenetic tree of Mesothelin



Discussion

Protein Analysis and statistics of alpha-fetoprotein

The number of basic amino acids in human Alpha-fetoprotein is 53 and acidic amino acids are 67. Number of buried amino acids in Alpha-fetoprotein is 203.Estimated Radius of Folded Protein 30.5495. Percentage of hydrophobic amino acids in human Alpha-fetoprotein is 47.7551. The molecular weight of human Alpha-fetoprotein is 55563.793.

Protein Analysis and statistic Mesothelin

The number of basic amino acids in human mesothelin is 63 and acidic amino acids are 70. Number of buried amino acids in mesothelin are 279. Estimated Radius of Folded Protein 33.0777. Percentage of hydrophobic amino acids in human mesothelin is 50.4823. The molecular weight of human mesothelin is 68010.656.

Conclusion

Alpha-fetoprotein

From the results and discussion of present *In-silico* protein analysis study of vertebrate it can be concluded that the amino acid sequences among different vertebrate species shows slight to moderate differences without affecting its functions. The function of Alpha-fetoprotein is immune-regulatory properties and or may influence cell proliferation and growth. It also can be concluded from the phylogenetic tree analysis that the Alpha-fetoprotein in different vertebrates also has strong to moderate sequence similarities among studied vertebrates.

Mesothelin

From the results and discussion of present *In-silico* protein analysis study of vertebrate, it can be concluded that the amino acid sequences among different vertebrate species shows slight to moderate differences without affecting its functions. The function of mesothelin is cell adhesion. It also can be concluded from the phylogenetic tree analysis that the mesothelin in different vertebrates also have strong to moderate sequence similarities among studied vertebrates.

Acknowledgement

We are thankful to the NCBI for permission to access the valuable data regarding study purpose. We are also thankful to Department of Zoology, SGBAU University Amravati and Research Centre, Department of Zoology, Jijamata Mahavidyalaya, Buldhana.

References

1. Blohm M, Vesterling-Horner D, Calaminus C, Gobel U. Alpha,-fetoprotein (afp) reference values in infants up to 2 years of age, 1997.
2. Corapçıoglu Funda, Turker Gülcan, Aydogan Ayşen, Sarper Nazan, Duman. Arısoy A. Engin, serum alpha fetoprotein levels in healthy full-term neonates and infants, 2004.
3. Corso BS. Christopher, Stubbs MS. Desmond, Lee Sang-Hun, Goggins Michael, Hruban Ralph, Hunt William, real-time detection of mesothelin in pancreatic cancer cell line supernatant using an acoustic wave immunosensor, 2006.
4. Jingyu Zhan, Dong Lin, Nathan Watson, Wai Kwan Tang *et al*., (2023) Structures of Cancer Antigen Mesothelin and Its Complexes with Therapeutic Antibodies
5. Lisa H Butterfield, James S Economou, in Gene Therapy of Cancer (Second Edition), (2002) DNA and Dendritic Cell-Based Genetic Immunization against Cancer
6. Sathyanarayana Bangalore, Hahn Yoonsoo, Patankar Manish, Pastan Ira and Lee. Byungkook Mesothelin, eocilin. Ster and Otoancorin are predicted to have super helical structures with ARM-type repeats 2008.
7. http://www.bioperal.org/wiki/FASTA.
8. http://www.NCBI.com
9. http://www.Wikipiedia.com