

Molecular Evolutionary Studies Of Epidermal Growth Factors Using Multiple Sequence Alignment Methods

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ABSTRACT:-

Epidermal growth factor (EGF) is a growth factor that stimulates cell growth, proliferation and differentiation by binding to its receptor EGFR. Human EGF is a 6045-Da protein with 53 amino acid residues having three intramolecular disulfide bonds. The phylogenetic analysis can therefore help in research studies to discover the protein manually using bioinformatics tools. In this paper the four different species nucleotide and amino acid sequence were analyzed along with their physiological and anatomical properties in order to build a phylogenetic tree and perform a multiple sequence alignment using clustalx. If so all given species have same nucleotide and amino acid sequence alignment along with their physiological and anatomical similarities they can be implemented for research studies.

INTRODUCTION

The epidermal growth factor receptor is the cell-surface receptor for members of the epidermal growth factor family (EGF-family) of extracellular protein ligands. The epidermal growth factor receptor is a member of the ErbB family of receptors, a subfamily of four closely related receptor tyrosine kinases: EGFR (ErbB-1), HER2/c-neu (ErbB-2), Her 3 (ErbB-3) and Her 4 (ErbB-4). Mutations affecting EGFR expression or activity could result in cancer [5].

Epidermal growth factor and its receptor was discovered by Stanley Cohen of Vanderbilt University. Cohen shared the 1986 Nobel Prize in Medicine with Rita Levi-Montalcini for their discovery of growth factors [4]. EGFR (epidermal growth factor receptor) exists on the cell surface and is activated by binding of its specific ligands, including epidermal growth factor and transforming growth factor α (TGF α) (note, a full list of the ligands able to activate EGFR and other members of the ErbB family is given in the ErbB article) [2, 3]. ErbB2 has no known direct activating ligand, and may be in an activated state constitutively or become active upon heterodimerization with other family members such as EGFR. Upon activation by its growth factor ligands, EGFR undergoes a transition from an inactive monomeric form to an active homodimer, although there is some evidence that preformed inactive dimers may also exist before ligand binding [12]. In addition to forming homodimers after ligand binding, EGFR may pair with another member of the ErbB receptor family, such as ErbB2/Her2/neu, to create an activated heterodimer. There is also evidence to suggest that clusters of activated EGFRs form, although it remains unclear whether this clustering is important for activation itself or occurs subsequent to activation of individual dimers [8, 14].

EGFR dimerization stimulates its intrinsic intracellular protein-tyrosine kinase activity. As a result, autophosphorylation of several tyrosine (Y) residues in the C-terminal domain of EGFR occurs. These include Y992, Y1045, Y1068, Y1148 and Y1173, as shown in the diagram to the left [1]. This autophosphorylation elicits downstream activation and signaling by several other proteins that associate with the phosphorylated tyrosines through their own phosphotyrosine-binding SH2 domains. These downstream signaling proteins initiate several signal transduction cascades, principally the MAPK, Akt and JNK pathways, leading to DNA synthesis and cell proliferation. Such proteins modulate phenotypes such as cell migration, adhesion, and proliferation. Activation of the receptor is important for the innate immune response in human skin. The kinase domain of EGFR can also cross-phosphorylate tyrosine residues of other receptors it is aggregated with, and can itself be activated in that manner [10,11,15].

Mutations that lead to EGFR overexpression (known as upregulation) or overactivity have been associated with a number of cancers, including lung cancer, anal cancers and glioblastoma multiforme. These somatic mutations involving EGFR lead to its constant activation, which produces uncontrolled cell division. In glioblastoma a more or less specific mutation of EGFR, called EGFRvIII is often observed [9,12]. Mutations, amplifications or misregulations of EGFR or family members are implicated in about 30% of all epithelial cancers [6, 7].

The identification of EGFR as an oncogene has led to the development of anticancer therapeutics directed against EGFR (called "EGFR inhibitors"), including gefitinib, erlotinib, afatinib, and icotinib for lung cancer, and cetuximab for colon cancer. Many therapeutic approaches are aimed at the EGFR. Cetuximab and panitumumab are examples of monoclonal antibody inhibitors. However the former is of the IgG1 type, the latter of the IgG2 type; consequences on antibody-dependent cellular cytotoxicity can be quite different. Other monoclonals in clinical development are zalutumumab, nimotuzumab, and matuzumab. The monoclonal antibodies block the extracellular ligand binding domain. With the binding site blocked, signal molecules can no longer attach there and activate the tyrosine kinase [12].

As Gefitinib is a selective chemotherapeutic agent, its tolerability profile is better than previous cytotoxic agents. Gefitinib inhibits EGFR tyrosine kinase by binding to the adenosine triphosphate (ATP)-binding site of the enzyme. Thus the function of the EGFR tyrosine kinase in activating the anti-apoptotic Ras signal transduction cascade is inhibited, and malignant cells are inhibited [23].

The EGF-like domain is an evolutionary conserved protein domain, which derives its name from the epidermal growth factor where it was first described. It comprises about 30 to 40 amino-acid residues and has been found in a large number of mostly animal proteins. Most occurrences of the EGF-like domain are found in the extracellular domain of membrane-bound proteins or in proteins known to be secreted. An exception to this is the prostaglandin-endoperoxide synthase [18]. The EGF-like domain includes 6 cysteine residues which in the epidermal growth factor have been shown to form 3 disulfide bonds. The structures of 4-disulfide EGF-domains have been solved from the laminin and integrin proteins. The main structure of EGF-like domains is a two-stranded β -sheet followed by a loop to a short C-terminal, two-stranded β -sheet. These two β -sheets are usually denoted as the major (N-terminal) and minor (C-terminal) sheets. EGF-like domains frequently occur in numerous tandem copies in proteins: these repeats typically fold together to form a single, linear solenoid domain block as a functional unit [19, 21].

Despite the similarities of EGF-like domains, distinct domain subtypes have been identified. The two main proposed types of EGF-like domains are the human EGF-like (hEGF) domain and the complement C1r-like (cEGF) domain, which was first identified in the human complement protease. The differentiation of cEGF-like and hEGF-like domains and their subtypes is based on structural features and the connectivity of their disulfide bonds. In conclusion, although distinct EGF-like domains are grouped, subtypes can be clearly separated by their sequence, conformation and, most importantly, their function [20, 22].

Materials and Methods :

NCBI – National Center for Biotechnology Information

The National Center for Biotechnology Information (NCBI) is part of the United States National Library of Medicine (NLM), a branch of the National Institutes of Health. The NCBI houses genome sequencing data in GenBank and an index of biomedical research articles in PubMed Central and PubMed, as well as other information relevant to biotechnology. All these databases are available online through the Entrez search engine. The NCBI houses genome sequencing data in GenBank and an index of biomedical research articles in PubMed Central and PubMed, as well as other information relevant to biotechnology. All these databases are available online through the Entrez search engine. It creates public databases, conducts research in computational biology, develops software tools for analyzing genome data, and disseminates biomedical information – all for the better understanding of molecular processes affecting the human health and diseases. It also serves as an available source for DNA sequences of various organisms to evolutionary biologists. Entrez is the integrated, text-based search and retrieval system used at NCBI for the major databases, including PubMed, nucleotide and protein sequences (and structures). NCBI fosters scientific communication by sponsoring meetings, workshops and lecture series as well as supports training on basic and applied research in computational biology. The NCBI has had the responsibility for making available the GenBank DNA sequence database since 1992. GenBank coordinates with individual laboratories and other sequence databases such as those of the European Molecular Biology Laboratory (EMBL) and the DNA Data Bank of Japan (DDBJ) [23-26].

BLAST – Basic Local Alignment Search Tool

BLAST, in bioinformatics, is an algorithm for comparing primary biological sequence information, such as the amino-acid sequences of different proteins or the nucleotides of DNA sequences. A BLAST search enables a researcher to compare a query sequence with a library or database of sequences, and identify library sequences that resemble the query sequence above a certain threshold. Different types of BLASTs are available according to the query sequences. For example, blastn for nucleotide sequences & blastp for protein sequences. It is one of the most widely used bioinformatics programs, because it addresses a fundamental problem and the algorithm emphasizes speed over sensitivity. This emphasis on speed is vital to making the algorithm practical on the huge genome databases currently available, although subsequent algorithms can be even faster. BLAST uses a heuristic method to find homologous sequences, not by comparing sequence in its entirety, but rather by locating short matches between the two sequences (this process is called seeding). It is after this first match that BLAST begins to make local alignments. It can be used in any operating systems such as UNIX, Linux, Mac and Windows.

Nucleotide and Protein BLAST (blastn)

This program, given a DNA query, returns the most similar DNA sequences from the DNA database that the user specifies. It is useful for possibly identifying a species and/or find homologous species. This program, given a protein query, returns the most similar amino acid sequences from the protein database that the user specifies. It is useful for possibly identifying a species and/or find homologous species.

CLUSTALX

Clustal X is a windows interface for the ClustalW multiple sequence alignment program. It provides an integrated environment for performing multiple sequence and profile alignments and analysing the results. The sequence alignment is displayed in a window on the screen. A versatile coloring scheme has been incorporated allowing you to highlight conserved features in the alignment. The pull-down menus at the top of the window allow you to select all the options required for traditional multiple sequence and profile alignment. Using Clustal X it is possible to cut-and-paste sequences to change the order of the alignment; you can select a subset of sequences to be aligned; you can select a sub-range of the alignment to be realigned and inserted back into the original alignment. Alignment quality analysis can be performed and low-scoring segments or exceptional residues can be highlighted. [27-32]ClustalX is available on Linux, Mac and Windows.

SEQUENCE INPUT

Sequences and profiles (a term for pre-existing alignments) are input using the FILE menu. Invalid options will be disabled. All sequences must be included into 1 file. 7 formats are automatically recognised: NBRF/PIR, EMBL/SWISSPROT, Pearson (Fasta), Clustal (*.aln), GCG/MSF (Pileup), GCG9 RSF and GDE flat file. All non-alphabetic characters (spaces, digits, punctuation marks) are ignored except "-" which is used to indicate a GAP ("." in MSF/RSF).

PHYLOGENETIC TREES

Phylogenetic trees can be calculated from old alignments (read in with "-" characters to indicate gaps) OR after a multiple alignment while the alignment is still displayed.

ALIGNMENT DISPLAY

The alignment is displayed on the screen with the sequence names on the left hand side. The sequence alignment is for display only, it cannot be edited here (except for changing the sequence order by cutting-and-pasting on the sequence names). A ruler is displayed below the sequences, starting at 1 for the first residue position (residue numbers in the sequence input file are ignored). A line above the alignment is used to mark strongly conserved positions.[33-36] Three characters ("*", ":" and ".") are used: "*" indicates positions which have a single, fully conserved residue. ":" indicates that one of the following 'strong' groups is fully conserved:

TREE VIEW:-

TreeView is a simple program for displaying phylogenies on Apple Macintosh and Windows PCs. It has the following features:

- runs on both the Apple Macintosh and Windows PCs, using almost identical interfaces
- reads many different tree file formats (including NEXUS, PHYLIP, Hennig86, NONA, MEGA, and ClustalW/X)
- supports standard the TrueType and Postscript fonts available on Macs and PCs
- supports native graphics file format (PICT on Macintosh, Windows metafile on Windows) for copying pictures into other applications, and for saving graphics files
- print preview
- can print multiple trees per page, and one tree over more than one page
- has drag-and-drop facility for easy opening of files
- Web-based online help
- tree editor

TreeView provides a simple way to view the contents of a NEXUS, PHYLIP, Hennig86, Clustal, or other format tree file. While PAUP and MacClade have excellent tree printing facilities, there may be times you just want to view the trees without having to load the data set they were generated from. The PHYLIP package contains tree drawing programs which offer a greater variety of trees than TreeView, but are somewhat clumsy to use. The forthcoming PAUP* for Windows does not have a graphical interface, hence TreeView allows you to create publication quality trees from PAUP files, either directly, or by generating graphics files for editing by other programs. The program currently reads trees with up to 1000 taxa. View the human EGF protein and nucleotide sequence in fasta format from NCBI database and run in Blastp and Blastn and get any four species with 100% similarities entries. Select the sequence and apply to Clustalx. Complete the phylogeny tree through complete Alignment and save the image in graphical format.[37-39]

Results and Discussion:

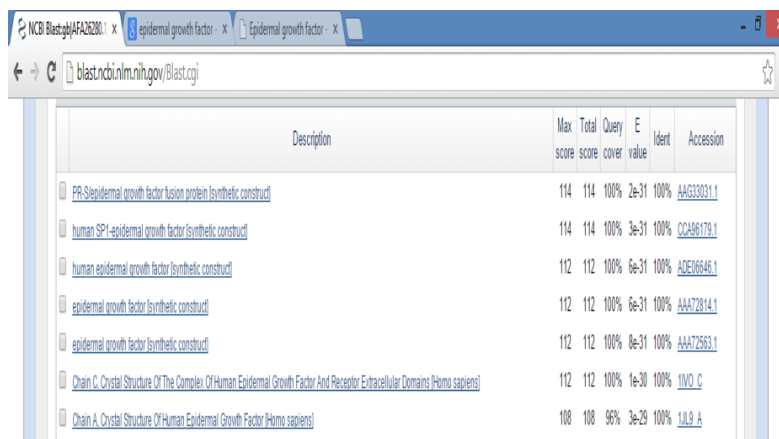
Clustal x has two modes which can be selected using the switch directly above the sequence display: multiple alignment mode and profile alignment mode. to do a multiple alignment on a set of sequences, make sure multiple alignment mode is selected. a single sequence data area is then displayed. the alignment menu then allows you to either produce a guide tree for the alignment, or to do a multiple alignment following the guide tree, or to do a full multiple alignment.

In profile alignment mode, two sequence data areas are displayed, allowing you to align 2 alignments (termed profiles). Profiles are also used to add a new sequence to an old alignment, or to use secondary structure to guide the alignment process. Gaps in the old alignments are indicated using the "-" character. profiles can be input in any of the allowed formats; just use "-" (or "." for msf/rsf) for each gap position. in profile alignment mode, a button "lock scroll" is displayed which allows you to scroll the two profiles together using a single scroll bar, while when the lock scroll is turned off, the two profiles can be scrolled independently. The search results for EGF from NCBI was shown in Figure 1 and the transcript variants

were shown in Figure 2. The amino acids sequence of multiple alignment mode through FASTA format was displaced in Figure 3 and finally the phylogenetic tree was shown in Figure 4.

From the result it is inferred that almost all protein sequence of those species are almost shows 100 % similarities and in case of nucleotide sequence pan troglodytes have 98% similarity and in case of pongo abelii has 97% similarity and in case of macaca mulatta has 95% similarity and macaca fascicularis has 95% similarity [23]. From this the multiple sequence alignment has been made. Finally the phylogenetic tree has been built which showed that macaca mulatta, homosapiens and pongo abelii have had the same ancestor. The macaca fascicularis, pan troglodytes are derived from the species pongo abelii [15]. Thus, the similarities between five species in terms of protein, amino acid sequence and the anatomical and physiological similarities and to align a multiple sequence alignment of those species and align with using clustalx and to build a phylogenetic tree of those species are found and build. Therefore if we want to do research studies in Epidermal growth factor we can use these species for research and if so there is low secretion of growth factor we can use these species sample for preparing growth factor manually using biotechnology techniques.[40-45]

Figure 1 : Blast NCBI search results for Epidermal Growth Factor



Description	Max score	Total score	Query cover	E value	Ident	Accession
PR-Siepidermal growth factor fusion protein (synthetic construct)	114	114	100%	2e-31	100%	AAG33031.1
human SP4-epidermal growth factor (synthetic construct)	114	114	100%	3e-31	100%	CC486179.1
human epidermal growth factor (synthetic construct)	112	112	100%	6e-31	100%	A0C06946.1
epidermal growth factor (synthetic construct)	112	112	100%	6e-31	100%	A3472814.1
epidermal growth factor (synthetic construct)	112	112	100%	8e-31	100%	A4472563.1
Chain C, Crystal Structure Of The Complex Of Human Epidermal Growth Factor And Receptor Extracellular Domains (Homo sapiens)	112	112	100%	1e-30	100%	1W0_C
Chain A, Crystal Structure Of Human Epidermal Growth Factor (Homo sapiens)	108	108	96%	3e-29	100%	1UL9_A

Figure 2 : Transcript variants of Human Epidermal Growth Factors

Sequence Description	Length	Query Length	Percentage	Score	E-value	Accession Number
PREDICTED: Chelonobius sabaeus epidermal growth factor receptor (EGFR) mRNA	872	872	13%	0.0	96%	XM_007981280.1
PREDICTED: Pan troglodytes epidermal growth factor receptor (EGFR) transcript variant X1, mRNA	726	726	10%	0.0	99%	XM_519102.5
PREDICTED: Pongo abelii epidermal growth factor receptor (EGFR) mRNA	669	669	10%	0.0	97%	XM_002817937.3
Homo sapiens genomic sequence surrounding Nci1 site, clone NL1-D011C	660	660	14%	0.0	88%	A1326052.1
Homo sapiens epidermal growth factor receptor (erythroid leukemia viral (v-erb-b) oncogene homolog, avian) mRNA (cDNA clone M)	638	638	8%	2e-178	100%	BC264761.1
Homo sapiens truncated epidermal growth factor precursor (EGFR) mRNA, complete cds	610	610	8%	5e-170	99%	AF125253.1
Human epidermal growth factor precursor (EGFR) mRNA, complete cds	608	608	8%	2e-169	99%	U48722.1
Homo sapiens epidermal growth factor receptor mRNA, complete cds, alternatively spliced	606	606	8%	7e-169	99%	H02912715.1
Homo sapiens epidermal growth factor receptor (EGFR) transcript variant 4, mRNA	606	606	8%	7e-169	99%	NM_201284.1
Homo sapiens epidermal growth factor receptor (EGFR) transcript variant 3, mRNA	606	606	8%	7e-169	99%	NM_201283.1

Figure 3: EGF amino acid sequence analysis using FASTA in multiple alignment mode

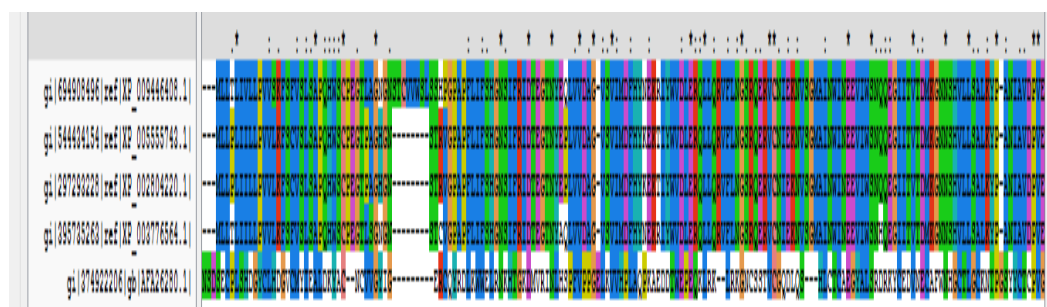
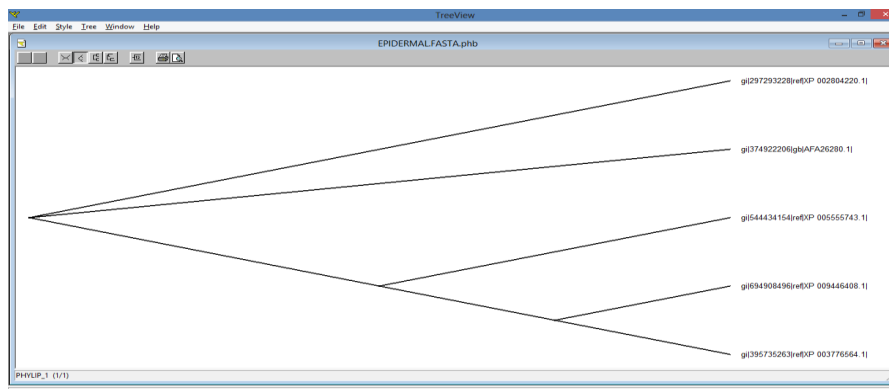


Figure 4 : Phylogenetic Tree developed for human EGF



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