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Comparative analysis of *Homo sapiens* ERBB2 erb-b2 receptor tyrosine kinase 2

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Abstract

Growth factors are special proteins which help to stimulate proliferation and differentiation in both normal and malignant cells. The first growth factor was epidermal growth factor. The receptor tyrosine kinases includes the receptor epidermal growth factor receptor EFGR. They have many other members like erbB2/HER-2, erbB3/HER-3, and erbB4/HER. These receptors are anchored in the cytoplasmic membrane and share a similar structure that is composed of an extracellular ligand-binding domain, a short hydrophobic transmembrane region, and an intracytoplasmic tyrosine kinase domain. Activation of these receptor leads to the phosphorylation of important tyrosine residues within the COOH-terminal portion of EGFR resulting in specific docking sites for cytoplasmic proteins. To overcome various problems associated with ERBB gene mutations, prior identification and analysis of these mutations is necessary. In this study, we have analyzed the ERBB receptor tyrosine kinase, its structural classification and various protein domains by using bioinformatics tools.

Keywords: Erbb3, Hydrophobic, Transmembrane region, Ligand, Domain.

Introduction

The ErbB family of receptor tyrosine kinases contains many growth factor receptors. Most of the epidermal growth factor receptor (EGFR) belongs to the family of receptors named, ErbB. The EGFR gene is in actual involved for providing instructions to make an epidermal growth factor receptor protein. Activation of transmembrane proteins is carried out by binding with peptide growth factors of the EGF-family of specialized proteins. The particular positioning of EFGR gene allows the receptor to bind with other some proteins like ligands. Ligand-receptor binding resembles to key and lock phenomena and get fixed in a same way. The epidermal growth factor receptor binds many different ligands. Out of several ligands at least seven different ligands were found with receptor binding.

It was observed that the EGFR becomes activated by receptor overexpression and due to some ligand attachment. Different number of ligands are found that bind to the EGFR, these are mainly six including EGF itself. According to previous studies, binding of ligand to a particular receptor induces a conformational change to the receptor external domain leading towards receptor dimerization and auto phosphorylation of certain tyrosine residues[1-2].

It was also previously reported that the ligand-independent receptor activation occurs in some tumors that display forms of the EGFR and HER having no extracellular domain[3-4]. Ligand independent activation of EFGR resulted because of prolinase-type plasminogen activator receptor overexpression activity via association with $\alpha_5\beta_1$ integrin[5-6].

Moreover, the activation of receptor independently of ligand resulted because of multiple cellular processes, mainly due to radiation. Due to this, phosphatases are silenced and helps to antagonize the receptor kinase activity, shifting the equilibrium of basal phosphorylation[7]. According to some studies, ErbB2 is found to be involved in activating signaling molecules which regulate the bioenergetic metabolism[8-13].

Mutation in EFGR: According to various studies, at least eight mutations in the EGFR gene have been associated with lung cancer. Nearly all these EGFR gene mutations are somatic and are only present in cancer cells. These mutations are most common in non-smokers. Amplification of the EGFR gene and its related mutations with tyrosine kinase domains been observed in many carcinoma patients. Mutations of somatic cells are found in four different exons within the kinase receptor domain. These mutations arise more frequently found in a subpopulation of non-small cell lung cancer patients[14,15].

On the other hand, it was noticed about the *K-RAS* mutations which are frequent in smokers, correlates with resistance to EGFR inhibitors. Therefore, the ERBB receptors dimerize on ligand binding and with other members of the ERBB family[16]. Hence, the excessive ERBB signaling is associated with the development of a wide variety of solid tumors and insufficient ERBB signaling in humans are associated with the development of neurodegenerative diseases[17-18].

Tyrosine Kinase Function: Protein tyrosine kinase receptors needs a coreceptor for ligand binding. It plays an important role as an essential component of a neuregulin-receptor complex. Studies suggested, GP30 as a potential ligand for this receptor. Also found to be involved in regulation and stabilization of certain peripheral microtubules. It was also found to function in the nucleus, transcriptional regulation and cellular growth mechanisms.

This study involves, in silico analysis of human ERBB gene with its similar variants and others from different species. The reported paralogs and orthologues was analyzed for further comparison. The protein domain for receptor tyrosine kinases was also observed by using various computational tools. The obtained analysis results are proposed with specific predictions.

Material and methods

Sequence analysis of human erbb2 TKI gene shown the coding region of gene. Various computational studies shown different protein domain present in homo sapiens erbb2 gene. Different bioinformatics software's were used to analyze the selected erbb2 gene and to compare this gene with its paralogs and orthologues.

HGNC reported symbol for ERBB2: The human ERBB2 gene was searched in Hugo Gene Nomenclature Committee.

Sequence retrieval of human ERBB2 gene: Sequence of erbb2 gene was retrieved from NCBI-Genbank data repository.

Transcript analysis: Transcript maps were obtained through Ensemble software.

Structural classification of ERBB2 protein domains: InterPro was used to study various protein matches and signatures.

Analysis of human ERBB2 protein Kinase domains: Protein domains were analyzed using EBI-PDB.

Results and discussion

Comparative study of homo sapiens erbb gene depicts its various paralogs and orthologues in different species. Results are shown in the tables below:

Table-1: Shows reported paralogs of human ERBB2.

Name	ID	Location	Description	Origin
EGFR	1956	Chromosome 7	epidermal growth factor receptor	*Homo sapiens*
ERBB2	2064	Chromosome 17	erb-b2 receptor tyrosine kinase 2	*Homo sapiens*
GRB2	2885	Chromosome 17	growth factor receptor bound protein 2	*Homo sapiens*
ERBB3	2065	Chromosome 12	erb-b2 receptor tyrosine kinase 3	*Homo sapiens*
ERBB4	2066	Chromosome 2	erb-b2 receptor tyrosine kinase 4	*Homo sapiens*
EPS15	2060	Chromosome 1	epidermal growth factor receptor pathway substrate 15	*Homo sapiens*
EPS8	2059	Chromosome 12	epidermal growth factor receptor pathway substrate 8	*Homo sapiens*
ESR1	2099	Chromosome 6	estrogen receptor 1	*Homo sapiens*
GRB7	2886	Chromosome 17	growth factor receptor bound protein 7	*Homo sapiens*

Table-2: Shows reported orthologues of human ERBB2.

Name	ID	Location	Description	Origin
ERBB2	2064	Chromosome 17	erb-b2 receptor tyrosine kinase 2	*Homo sapiens*
Erbb2	13866	Chromosome 11	erb-b2 receptor tyrosine kinase 2	*Mus musculus*
Erbb2	24337	Chromosome 10	erb-b2 receptor tyrosine kinase 2	*Rattusnorvegicus*
erbb2	386966	Chromosome 12	erb-b2 receptor tyrosine kinase 2	*Danio rerio* (zebrafish)
ERBB2	403883	Chromosome 9	erb-b2 receptor tyrosine kinase 2	*Canis lupus familiaris*
ERBB2	697573	Chromosome 16	erb-b2 receptor tyrosine kinase 2	*Macacamulatta*
ERBB2	505709	Chromosome 19	erb-b2 receptor tyrosine kinase 2	*BosTaurus*
ERBB2	454636	Chromosome 17	erythroblastic leukemia viral oncogene homolog 2	*Pan troglodytes*
ERBB2	426130	Chromosome 27	erythroblastic leukemia viral oncogene homolog 2	*Gallus gallus*
ERBB2	100054739	Chromosome 11	erb-b2 receptor tyrosine kinase 2	*Equuscaballus*

Chromosome Map: The erbb2 gene was found to be located on long arm of chromosome 17 at position 12. The analysis shows gene is located from base pair 39,688,084 to 39,728,662 on chromosome 17 (Figure-3).

Transcript analysis: The transcript with their specific Ensemble Id are represented in Figure-4.

APPROVED SYMBOL	ERBB2
APPROVED NAME	erb-b2 receptor tyrosine kinase 2
HGNC ID	HGNC:3430
PREVIOUS SYMBOLS & NAMES	NGL, "v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 2", "v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 2 (neuro/glioblastoma derived oncogene homolog)"
SYNONYMS	CD340, HER-2, HER2, "human epidermal growth factor receptor 2", NEU, "neuro/glioblastoma derived oncogene homolog"
LOCUS TYPE	gene with protein product

Figure-1: Shows the reported erb-b2 receptor tyrosine kinase 2

AAGACAAATGGGCGGGGCTTACCGTGGGGGTGGGGCTTACCTGCTAGACTCATGTAGATTGGCTGGCGGGAGCGGAAGTGATTCAGAGCGCCCCCAGAGCAGTTCTGCTCTTCGC
ACTGCAGTACGCAGTCGCGGTACACCGGCTCACGGTCGCCCTGGGAGCCGCTCGCCAGCGCCGCTGCCCCAGCTAGCAGGACCAACCGCGCCGCCAGGCCGGCCATCCTTTCTCC
CTGGCTCGCCGCCGGGGGAGGAGCTTAGGAGTATGAAGCTTCCACTTCCGGAGTAACCGGAAGTTCCTGTGTTCTTTATTCTACTCTCCGCTGAAGTCCACACAGTTTAAATTAA
AGTTCCCGGATTTTTGTGGGCGCCTGCCCCGCCCCTCGTCCCCCTGCTGTGTCCATATATCGAGGCGATAGGGTTAAGGGAAGGCGGACGCCTGATGGGTTAATGAGCAAACTGA
AGTGTTTTCCATGATCTTTTTTGAGGTAGGGCTGTTTACTGTCACCACCCCTGTCGGATTTTACTTCCTAAACGTACCTGTAACTATCCACTTCTCTCCATCTCTTCTGGCACCA
CCCTGGTTAAAGACACCATCATGTGTCGCCAAGACAGCCGCAGTAGCTTCTTAATGGCTCTCCCTGCCTCTACTTTTGCCTCTTCCAACCTGCGCTCCATTTTGAAAAATTAAAA
TTTGCCCATATCACTTTTTTTTTCTTAAAATTATTTACTGGCTCCCAATTACCTTGGGTAAAATACAGTCTCCACAAACCCTGCCTGATTTGGCCCCTGTCCACTGGTCTCCCTC
ACTCCCTTGCTCCAGACCCGCTTCAGAGGGCTATGTCCCTCAAGCTTCCTGACTGCCTGGCCTGGTCTGAATCACTCACTCTTCTTTTTTCTTCTAGTCGCAATTGAAGTACCAC
CTCCCGAGGGTGATTGCTTCCCCATGCGGGGTAGAACCTTTGCTGTCCTGTTCACCACTCTACCTCCAGCACAGAATTTGGCTTATGGTAGGCGCTAACTGCGTTTGTTTGTTCT
TCTGTTTAATGAATGAACAGCATACATCAACATAAGAACTTGACAAATCCAGGGCTGTAAAATCATCAGTATGGTTCTGCACTGAGATCGGAGAGAAGTAA

Figure-2: Sequence of human erb-b2 receptor tyrosine kinase 2. The highlighted area shows the exons.

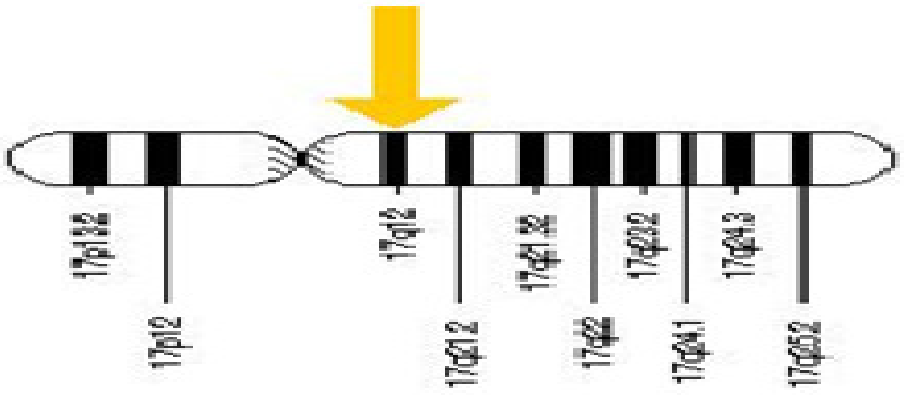

Figure-3: Shows the chromosome map of human ERBB2 gene.

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	RefSeq	Flags
ERBB2-001	ENST00000584601	4792	1225aa	Protein coding	CCDS45667	P04626	NM_001005862 NP_001005862	TSL:2 GENCODE basic
ERBB2-201	ENST00000406381	4564	1225aa	Protein coding	CCDS45667	P04626	-	TSL:5 GENCODE basic
ERBB2-008	ENST00000269571	4545	1255aa	Protein coding	CCDS32642	P04626 X5DNK3	NM_004448 NP_004439	TSL:1 GENCODE basic APPRIS P1
ERBB2-202	ENST00000541774	4341	1240aa	Protein coding	CCDS74052	P04626	NM_001289936 NP_001276865	TSL:5 GENCODE basic
ERBB2-005	ENST00000584450	3730	1055aa	Protein coding	CCDS77016	J3QLU9	NM_001289937 NP_001276866	TSL:1 GENCODE basic
ERBB2-003	ENST00000578199	2526	603aa	Protein coding	CCDS77017	F5H1T4	NM_001289938 NP_001276867	TSL:1 GENCODE basic
ERBB2-004	ENST00000445658	3238	979aa	Protein coding	-	B4DTR1	-	TSL:2 GENCODE basic
ERBB2-018	ENST00000580074	754	251aa	Protein coding	-	J3KTI5	-	CDS 5' and 3' incomplete TSL:3
ERBB2-013	ENST00000584099	583	139aa	Protein coding	-	J3KS21	-	CDS 3' incomplete TSL:4
ERBB2-012	ENST00000578709	559	102aa	Protein coding	-	J3QLV2	-	CDS 3' incomplete TSL:4
ERBB2-019	ENST00000582818	529	177aa	Protein coding	-	J3QRJ7	-	CDS 5' and 3' incomplete TSL:3
ERBB2-015	ENST00000578502	497	166aa	Protein coding	-	J3QRX1	-	CDS 5' and 3' incomplete TSL:5
ERBB2-007	ENST00000578373	4523	90aa	Nonsense mediated decay	-	J3QL83	-	TSL:1
ERBB2-011	ENST00000582648	1013	96aa	Nonsense mediated decay	-	J3KRI9	-	TSL:5
ERBB2-020	ENST00000584888	500	No protein	Processed transcript	-	-	-	TSL:3
ERBB2-010	ENST00000583038	4930	No protein	Retained intron	-	-	-	TSL:2
ERBB2-006	ENST00000582788	2004	No protein	Retained intron	-	-	-	TSL:5
ERBB2-009	ENST00000584908	1316	No protein	Retained intron	-	-	-	TSL:1

Figure-4: Shows different Transcripts of ERBB2 with their ensemble ID's.

Table-4: Shows the human erbB2 receptor tyrosine kinase with accession ID P04626.

Receptor tyrosine-protein kinase erbB-2 (P04626)

Accession	P04626 (ERBB2_HUMAN)
Species	Homo sapiens (Human)
Length	1,255 amino acids (complete)

Sequence analysis and classification: The InterPro software was used to study various protein domain and signatures in the selected erbb TKI. The results are shown in Figure-5.

Analysis of human ERBB2 protein Kinase domains: The analysis of homo sapiens erbb2 protein domains was performed by using EBI-PDB. Results shown number of different configuration domains (Figure-6).

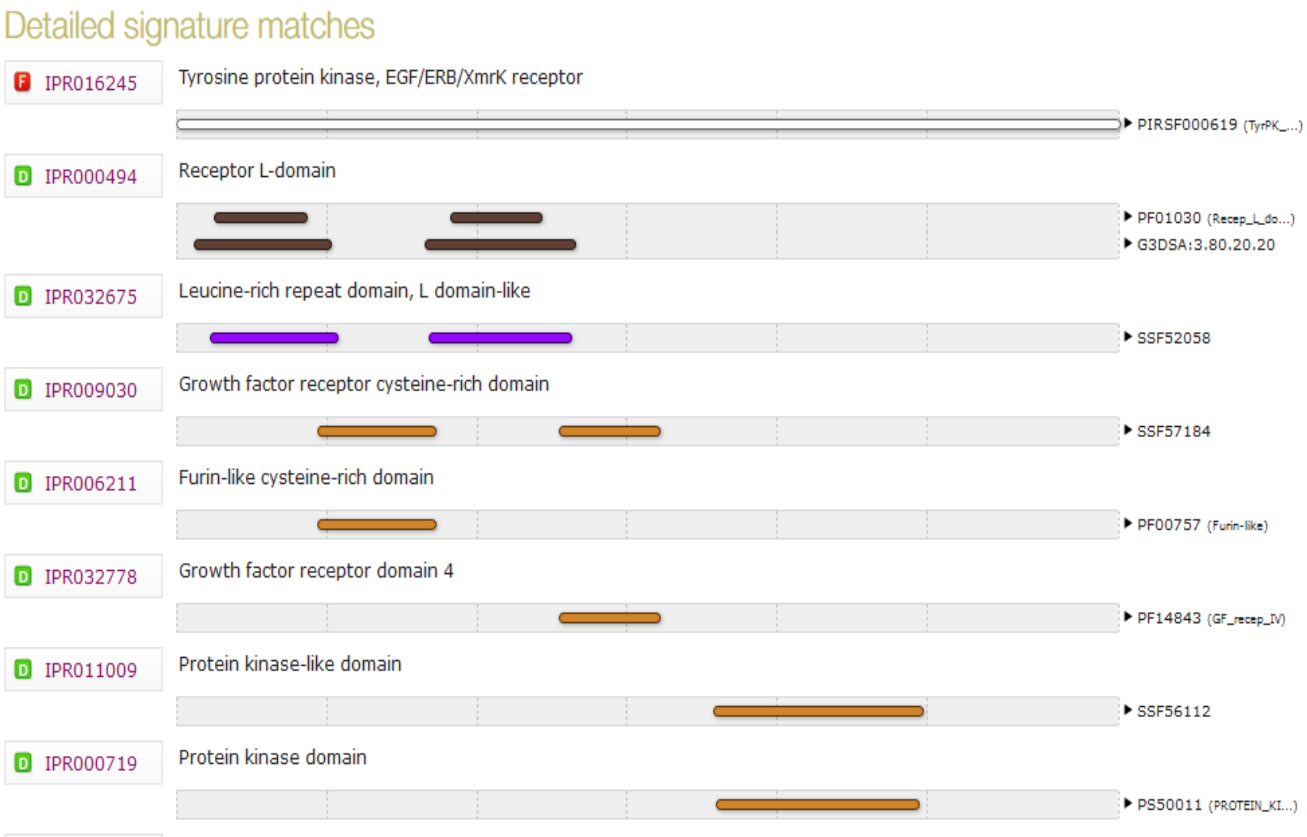

Figure-5: Shows erbb2 protein kinase domain obtained from InterPro Protein sequence analysis and classification.

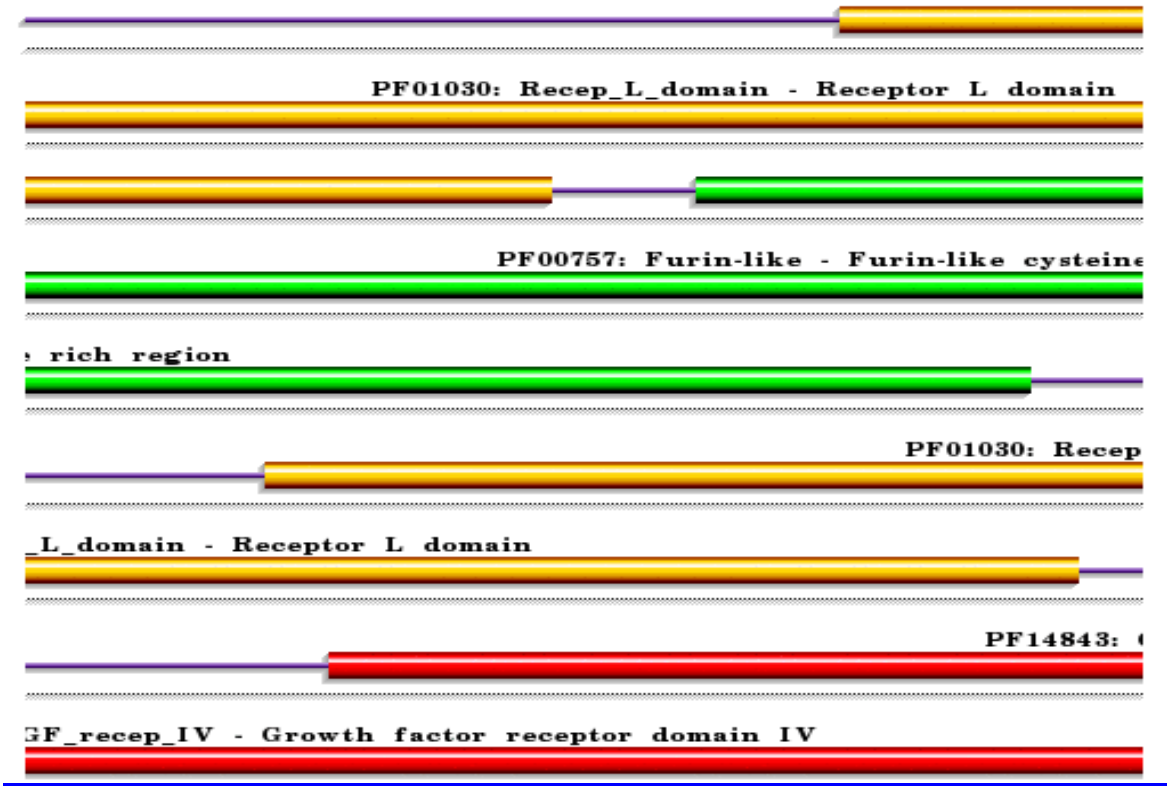

Figure-6: Shows various domains obtained from PDB. DB ID: 3pp0.

Discussion: The epidermal growth factor receptor works to induces cell differentiation and proliferation upon activation with ligand binding. The receptor is found at outer surface of cell which allows ligand and activates particularly the tyrosine kinase of receptoron its inner side. This tyrosine kinase helps to phosphorylates a variety of intracellular substrates that activates pathways leading to cellular growth and synthesis of DNA. According to some previous studies, overexpression of ErbB-2 in tissue culture ultimately leads to transformation and sometimes towards metastasis[19,20].

Conclusion

These findings implicate ErbB-2 as a major player in initiation and progression of breast cancer. The ErbB family of receptors are outstanding targets for breast cancer therapies and diagnosis. The analysis of ERBB gene by using bioinformatics tools can help to identify various gene variants within species by gene duplication events and in different species by speciation. Also, they can be useful for proper prediction of various putative domain in protein tyrosine kinase receptors.

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