Life Science Informatics Publications

Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences

Journal Home page http://www.rjlbpcs.com/

RJLBPCS

ISSN 2454-6348

**Original Research Article** **DOI: 10.26479/2019.0506.09**

# STRUCTURAL, FUNCTIONAL AND EVOLUTIONARY INSIGHT INTO NEUROPILIN-1 b1 DOMAIN

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**ABSTRACT:** Neuropilin (NRP) is a single transmembrane bound co-receptor for both vascular endothelial growth factor (VEGF) and semaphorin family members, found in vertebrates to function in axon guidance, angiogenesis, cell adhesion and wound healing. To gain insight into the structure function relation and evolutionary significance, we have analyzed the amino acid sequence of NRP1 from 29 species, its secondary structure and phylogenetic tree is computed. Structural analysis on VEGF-A165 binding region of NRP1 namely FAV/VIIIC domain (b1 domain) between zebrafish *Danio rerio* and human *Homo sapiens* using bioinformatics analysis like Clustal W, MEGA 6.0 and Swiss modular reveal their functional similarity mainly during angiogenesis. Further, we have studied zebrafish b1 domain in detail and compared with human to trace the angiogenic patterning between them. Strikingly conservation in primary, secondary and domain structures along with significant evolutionary conservation and similarity in b1 binding domain between zebrafish *Danio rerio* and human *Homo sapiens* suggesting extreme functional similarity exist and hence similar angiogenic role played by NRP1 during VEGF-A165 signaling. It is interesting to note that human NRP1 shows less homology with chimpanzee *Pan troglodytes*, gorilla *Gorilla gorilla*. Computed three-dimensional structural of zebrafish NRP1a-b1 domain exhibited very similar dissorted jelly $\beta$ barrel structure like-in humanNRP1 with high amino acids conservation and structurally similar VEGF-A165 binding loops and clefts. Suggesting zebrafish can be regarded as a potential model organism to understand the functional role played by NRPs in angiogenesis related studies. **Keywords:** Neuropilin-1; Angiogenesis; VEGF-A165; FAV/VIIIC b1 domain; Homology Modelling.

**Article History: Received: October 10, 2019; Revised: November 13, 2019; Accepted: December 02, 2019.**

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## 1.INTRODUCTION

Neuropilin (NRP), vertebrate specific membrane multi-domain protein made of 130 kDa (923 amino acids). The two neuropilins, NRP1 and NRP2 play versatile roles in angiogenesis, axon guidance and lymphangiogensis respectively [1], [2]. It has specific and overlapping expression patterns in several tissues. Their functions have been linked to their binding partners: semaphorins/collapsins (SEMA), vascular endothelial growth factors (VEGFs), fibroblast growth factors (FGFs), hepatocyte growth factor/scatter factor and heparin/heparin sulfate (HS) [3]–[5] . Multiplicity of ligands alongside complex formation with several membrane receptors makes neuropilins potential 'Signalling Hub' [4], [6], [7].

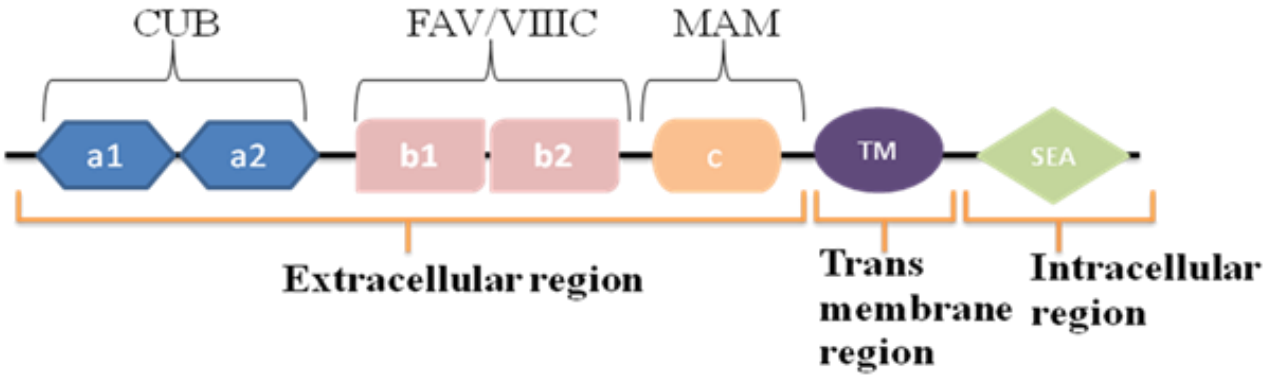



**Figure 1: Schematic representation of NRP1 domain structure.** Three regions-Extracellular region (divided into three unique domains: CUB domains or a1/a2 domain; FAV/VIIIC domains or b1/b2; MAM or c domain), Transmembrane region and Intercellular region (with SEA motif).

NRP1 structure (Figure 1) consists of three regions: extracellular region, transmembrane region and intercellular region [1], [8]. The large extracellular (EC) region with approximately 840 amino acids is subdivided into three unique domains. A two CUB or a1/a2 domain (110 F24-C275 amino acids), two FAV/VIIIC or b1/b2 domain (150 M276-A586 amino acids) and MAM or c domain (170 G647-C809 amino acids). CUB and FV/VIIIC domains are involved in VEGF and SEMA ligand binding and signaling, while MAM domain mainly contribute to the affinity for NRP [3], [9], [10] . The transmembrane (TM) region (24 I259- Y880 amino acids) is involved in receptor dimerization [11]. The intracellular or cytoplasmic (CYTO) region (43 C881-A923 amino acids) contains a short conserved Serine (S) - Glutamic acid (E) - Alanine (A) motif or PDZ binding domain [12]. NRP1 homologues have been identified so far in zebrafish, frog, chick, mouse, rat, human and other vertebrate animals, while it was not reported in invertebrates. In mutant mouse study, NRP1 was shown as essential for neuronal, angiogenic and cardiovascular development, whereas NRP2 has a more restricted role in neuronal patterning and lymphangiogenesis [9], [13], [14]. Similarly in zebrafish, morpholino knockdown of NRP1 in embryonic stage produces a severe vascular phenotype characterized by a loss or anarchic sprouting of new capillaries from pre-existing

intersomitic vessels [15]. From previous studies, NRP1 expression was crucial for the early embryonic segregation of arterial and venous cells in mammalian embryos [16], although the relatively with NRP2 deficient mice study, suggest that NRP2 expression may be less important in angiogenesis [17], [18]. To study the functional significance of NRP1a in vertebrates, secondary structure prediction, homology modelling was performed and the evolutionary significance was analysed by constructing a phylogenetic tree. Previous studies have signified the importance of this NRP1 domain in angiogenesis [3], [4], [19] and it was therefore our interest to study if the angiogenic pattern is conserved between human and zebrafish and if conserved, zebrafish could be used as a model for studying angiogenesis. Accordingly, zebrafish *Danio rerio*, NRP1a-b1 domain's 3D structure was modelled and compared with human *Homo sapiens* NRP1 to analyse the structural similarity between the two proteins. Our sequence analysis has demonstrated a strong conservation in primary structure of NRP1. Among divergent vertebrate and evolutionary conservation from lower vertebrate to higher species, indicating its importance in animal development. Structure comparison between human *Homo sapiens* NRP1 b1 with zebrafish *Danio rerio* shows high structural similarity, suggesting a similar function in both species. Hence NRP1 zebrafish *Danio rerio* may be used as a better *in-vivo* model system for the functional analysis of NRP1b in angiogenic signaling pathway.

## 2. MATERIALS AND METHODS

### Data mining and sequence assembly

Searches were performed using NRP protein sequences from Uniport. Sequences were carefully mined for completely available sequence, followed with its continuous usage by other researches as references in published journal. Isoforms for zebrafish have also been selected for the study. It has been found that only zebrafish contains 2 isoforms for each subclasses of NRP and it was also found to have number of soluble forms playing a major role in controlling and maintaining signals. Hence, the soluble isoforms has been excluded from this study. Individual protein sequences of NRP1 are retrieved in FASTA format for analysis. Sequence ID, scientific name of aminoacids in 15 vertebrate species includes are follows respectively. O14786 *Homo sapiens*, H2Q1T2 *Pan troglodytes*, F6UNY1 *Canis lupus familiaris*, E1BMX5 *Bos tarus*, W5Q7S8 *Ovis aries*, K7GQ07 *Sus scrofa*, P97333 *Mus musculus*, Q9QWJ9 *Rattus norvegicus*, H0VDN9 *Cavia porcellus*, G1SM30 *Oryctolagus cuniculus*, F1N8P1 *Gallus gallus*, H0YRS9 *Taeniopygia guttata*, P28824 *Xenopus laevis*, A4III3 *Xenopus tropicalis*, Q8QFX6 *Danio rerio* 1a, Q69DB7 *Danio rerio* 1b. These sequences were used for systematic interrogation and MSA interpretations.

### Multiple Sequence analysis

Multiple sequence alignment analysis was determined using Clustal Omega version tool supported by EMBL-EBI (European Bioinformatics Institute) and output file were collected. Retrieved FASTA sequence were globally aligned using Clustal tool with default parameters as non-identical

amino acid sequence with gap and BLOSSUM 6 algorithmic score was used to calculate matrix. Complete sequence alignment (data not shown) and percentage identity matrix output results were retrieved and used for the future analysis.

**Secondary structure prediction**

Secondary structure of NRP1 was predicted using GOR4 tool and predicted structures were aligned. GOR4 is a free user friendly tool which is available online. Individual FASTA sequence of NRP1 protein sequences was run on the program online. The obtained results were represented as Alpha helix (Hh) or α helix, Extended strand (Ee) or β sheets and Random coil (Cc) and in other secondary structures like $3_{10}$ helix (Gg), Pi helix (Ii), Beta bridge (Bb), Beta turn (Tt), Bend region (Ss), Ambiguous states and other states were predicted for the given sequence. Predicted structures are aligned in 100 scales of aminoacids and further structural analysis was done.

**Phylogenetic analysis**

Phylogenetic and molecular evolutionary analyses were conducted suing MEGA version 6 [20]. Primary sequence of 31 different species were computed with bootstrapped tree were built with 1000 replicates with the Maximum parsimony (MP) method with Poisson-corrected distances on amino acids; amino acid sites with gaps in any sequence were excluded from the calculations. Along with pervious list of species, few more species are also included for phylogenetic analysis to provide more evolutionary detail of NRP1. Common name, scientific name, accession number and number of amino acids in protein sequence are as follows respectively in Table.1

**Table 1. Animals used in study and their accession numbers:** Common name, species name, accession number, and length of vertebrate Neuropilin 1(NRP1) proteins used in analyses

| S.No. | Common name | Species name | NRP1accession No. | NRP1 length |
|---|---|---|---|---|
| 1 | Human beings | *Homo sapiens* | O14786 | 923 |
| 2 | Chimpanzee | *Pan troglodytes* | H2Q1T2 | 923 |
| 3 | Dog | *Canis familiar* | F6UNY1 | 925 |
| 4 | Bovine | *Bos tarus* | E1BMX5 | 926 |
| 5 | Sheep | *Ovis aries* | W5Q7S8 | 924 |
| 6 | Pig | *Sus scrofa* | K7GQ07 | 923 |
| 7 | Mouse | *Mus musculus* | P97333 | 923 |
| 8 | Rat | *Rattus norvegicus* | Q9QWJ9 | 922 |
| 9 | Guinea pig | *Cavia porcellus* | H0VDN9 | 927 |
| 10 | Rabbit | *Oryctolagus cuniculus* | G1SM30 | 905 |
| 11 | Chicken | *Gallus gallus* | F1N8P1 | 914 |
| 12 | Zebra finch | *Taeniopygia guttata* | H0YRS9 | 917 |
| 13 | Western clawed frog | *Xenopus laevis* | P28824 | 928 |
| 14 | African clawed frog | *Xenopus tropicalis* | A4III3 | 925 |

| | | | | |
|---|---|---|---|---|
| 15 | Zebra fish | *Danio rerio* (NRP1a) | Q8QFX6 | 923 |
| | | *Danio rerio* (NRP1b) | Q69DB7 | 959 |
| 16 | Medaka fish | *Oryzias latipae* | H2LIV8 | 922 |
| 17 | Japanese puffer fish | *Takifu rubripes* | H2S214 | 915 |
| 18 | West Indian ocean coelacanth | *Latimeria chalumnae* | H2ZYK3 | 916 |
| 19 | Chinese soft shell tutrle | *Pelodiscus sinesis* | K7FVI9 | 916 |
| 20 | Turkey | *Meleagris gallopava* | G1N330 | 893 |
| 21 | American chameleon | *Anolis carolinensis* | G1KFQ6 | 840 |
| 22 | Duck bil platypus | *Ornithorhynchus anatinus* | F7CIA9 | 920 |
| 23 | Horse | *Equus caballus* | F6T858 | 922 |
| 24 | African elephant | *Loxodonta africana* | G3SRK3 | 921 |
| 25 | Small eared galago | *Otolemur garnetii* | H0X2D1 | 926 |
| 26 | White tufted ear marmoset | *Callithrix jaccluus* | F6YYL6 | 928 |
| 27 | Rhesus macaque | *Macaca mulatta* | F6WJ50 | 906 |
| 28 | Sumatran orangutan | *Pongo abelli* | H2NA53 | 923 |
| 29 | Northern white cheeked gibbon | *Nomascus leucogenys* | G1S947 | 922 |
| 30 | Gorilla | *Gorilla gorilla* | G3QGL8 | 923 |
| 31 | 13 lined ground squirrel | *Spermophilus tridecemlineatus* | I3ML37 | 876 |

## 3. RESULTS AND DISCUSSION

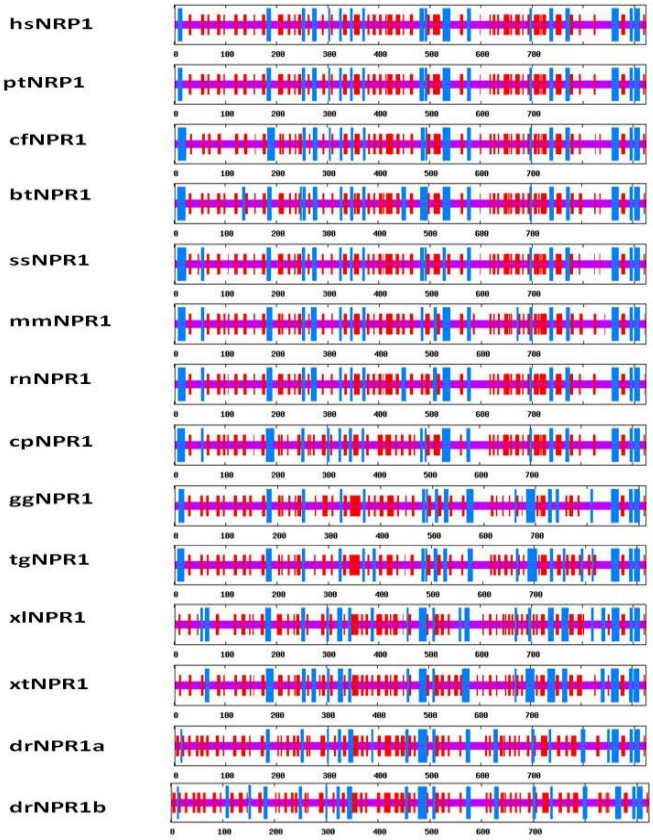
### Sequencing, Assembly and Alignment

The NRP1 full-length primary sequence of 15 vertebrate model organism including human, chimpanzee, dog, bovine, sheep, pig, mouse, rat, guinea pig, rabbit, zebra finch, western and African clawed frog and also zebrafish (a and b) were retrieved from Uniprot. Multiple sequence alignment (MSA) of NRP1 from various species revealed a high sequence homology among the various representative animal classes like chordate, aves and mammals. The N-ter region with the signaling peptide shows high diversity among the analyzed sequence. However, the three domain region namely EC, TM and CYTO domain of NRP1 shares a high degree of homology across species. This sequence conservation in various classes of animals indicates the significance of the receptor function for its survival. MSA analysis was carried out for different regions of NRP1, co-receptor such as like signal peptide; a1 and a2 domains; b1 and b2 domains; b/c linker region; c domain; c/TM linker; TM and CYTO. An in-depth analysis of the signal peptide region of all the species showed low sequence similarity, except rabbit *Oryctoagus cuniculus* which completely lacked the signal peptide. Based on the signal peptide conservation, the sequences have been classified into 2 groups; first being mammals and second aves, amphibians and chordate. The extracellular region harbours the ligand binding domain shows significant homology. It is also

observed that the b1/b2 domain exhibits greater sequence similarity than a1/a2 region. Among b1 and b2, b1 shows higher homology in selected species. TM region and CYTO region are highly conserved around 95%, depicting the structural and functional importance of these regions. Glycosylation site, N150, N269, N522 and S612 are highly conserved. In the overall sequence, a total of 19 cysteines present in NRP1 sequence and all were conversed in selected animal models and these cysteines could be essential to stabilize the secondary and tertiary structure of the protein. There is a SEA motif at C-ter considered to be an active part of the co-receptor further triggers signalling pathway [21]. This SEA motif is essential for NRP dependent endothelial cell migration and adhesion too, and found to be highly conserved. Interestingly, the linker regions which connect a/b, b/c, c/TM regions show significant sequence variations.

**Secondary structure analyses**

Secondary structure prediction of NRP1 (Figure 3) with GOR4 reveals the presence of approximately 15% to 20% of Alpha helix (Hh) or α helix, 25% to 30% of Extended strand (Ee) or β sheets and 55% to 59% of Random coil (Cc). Other secondary structures like $3_{10}$ helix (Gg), Pi helix (Ii), Beta bridge (Bb), Beta turn (Tt), Bend region (Ss) were absent. Chicken *Gallus gallus gallus* shows highest and western clawed frog *Xenopus leavis* shows the least coiled structure among all animals. Nrp has a higher percentage of β sheets, when compared to α helix. Cc, which are seen to connect β sheets and/or α helix or linker region are predominant. From the results obtained all mammals, aves, amphibians and zebra fish *Danio rerio* exhibits identical secondary structure at similar position. Our analysis reveals that the signal peptide in all the species forms an alpha helix except frogs which lack it completely and zebrafish in which only a few amino acids are involved. It is observed that majority of the secondary structure elements are conserved through all the species analysed, except slight variations in the position and number of amino acids. Zebrafish isoform drNRP1b shows additional alpha helix around $100^{th}$ and $150^{th}$ position. The ligand (VEGF) binding domain FAV/VIIIC between 300-400 positions shows similar pattern of alpha helix. However, subtle differences are observed in mammals, aves, reptiles and amphibians. Similary the b2 domain of FAV/VIIIC responsible for SEMS binding also shows conservation of alpha helices. Around $600^{th}$ position, unique alpha helix region is present only in zebrafish isoform drNRP1a and drNRP1b. The region around $700^{th}$ to $800^{th}$ amino acids shows conservation in mammals, aves and frog. A predominant helix at $700^{th}$ position is present zebrafish, frogs and aves. This helical region is absent in mammals, hinting the possibility that this helix could have some evolutionary significance. The regions at 870, 890 and $910^{th}$ position also shows well conserved 3 helices and this belongs to the intercellular region of the receptor.




| Organisms | Alpha helix (Hh) | Extended Strand (Ee) | Random coil |
|---|---|---|---|
| hsNRP1 | 140 is 15.17% | 249 is 26.98% | 534 is 57.85% |
| ptNRP1 | 140 is 15.17% | 250 is 27.09% | 523 is 57.75% |
| cfNRP1 | 149 is 16.11% | 246 is 26.59% | 530 is 57.30% |
| btNRP1 | 162 is 17.49% | 244 is 26.35% | 520 is 56.16% |
| ssNRP1 | 137 is 14.84% | 253 is 27.41% | 533 is 57.75% |
| mmNRP1 | 153 is 16.58% | 245 is 26.54% | 525 is 56.88% |
| rnNRP1 | 154 is 16.70% | 244 is 26.46% | 524 is 56.83% |
| cpNRP1 | 146 is 15.75% | 249 is 26.86% | 532 is 57.39% |
| ggNRP1 | 148 is 16.19% | 219 is 23.96% | 547 is 59.85% |
| tgNRP1 | 153 is 16.68% | 237 is 25.85% | 527 is 57.47% |
| xlNRP1 | 184 is 19.83% | 252 is 27.16% | 492 is 53.02% |
| xtNRP1 | 177 is 19.14% | 240 is 25.95% | 508 is 54.92% |
| drNRP1a | 140 is 15.17% | 260 is 28.17% | 523 is 56.66% |
| drNRP1b | 149 is 15.84% | 270 is 28.95% | 540 is 55.31% |

**Figure 3: a) Secondary structure aligned; b) Structural percentage of secondary structure.**

Using GOR4 tool secondary structure is predicted and aligned. Representative Alpha helix (Hh) or α helix, Extended strand (Ee) or β sheets and Random coil (Cc) and secondary structures aligned are scaled in 100 of aminoacids sequences and respective percentage was tabulated.

In NRP, approx. 29% of receptor is made of extended strand (β sheet). Among all animals, zebrafish shows highest percentage of extended strand. In inspection of extended strand, reveals that mammals and birds shows similar conserved patterns while frogs and zebrafish fall-out. On comparison of human and zebrafish around 200$^{th}$ to 400$^{th}$ position, similar numbers of strands are observed, despite the number of aminoacid. A big extended strand is seen at 500$^{th}$ position in mammals while it is replaced by smaller strands in aves, frogs and zebrafish. At 700$^{th}$ position, clustered extended strands are observed except in frogs and zebrafish. On overall analysis, the secondary structures like alpha helix and extended strands are highly conserved at the ligand binding regions indicating the functional similarity exhibited by these proteins.

**Molecular Evolution of NRP1**

A phylogenetic tree were constructed for all 31species, following multiple sequence alignment by Clustal W. Organisms with complete protein sequences (Ref Seq) were chosen for the study. The result of phylogenetic analysis (Figure 4) branches into two; the first branch consisting of aquatic animal, semi-aquatic, reptile, birds and rodent while second branch consists of mammals including both modern and archaic humans and other primates. From the phylogenetic tree (Figure.4), the

branch with more than 50% bootstrap value are considered for proving their correctness. Zebrafish, which belongs to the class of ray finned fishes, is one of the early ancestors for NRP1 along with medaka and puffer fish. Interestingly zebrafish is the only organism knows to have two isoforms of NRP1 because of an extra round of gene duplication and therefore there are two NRPs for every one NRP found in higher vertebrates. Aquatic clade includes Japanese puffer fish *Takifu rubripes*, zebra fish *Danio rerio*, medaka fish *Oryzias latipae*, western and African clawed frog *Xenopus laevis* and *Xenopus tropicalis* with high bootstrap value another clade includes birds like chicken *Gallus gallus*, turkey *Meleagris gallopava*, zebra finch *Taeniopygia guttata* along with reptiles like chinese soft shell turtle *Pelodiscus sinesis* and american chameleon *Anolis carolinensis*. Primates are grouped together signifying a common ancestor origin. A remarkable feature of the phylogenetic evaluation is the grouping of african elephant, small eared galago, 13 lined squirrel and dog distinctly. Interestingly this grouping could explained based on the axon guidance activity of NRP1 in the sensory organs [22], [23]. Sharp neuronal activity was required for a sharp memory and strong sense of smell. These four distinct species display one of these characters and hence outgroup the mammalian clade. Further analysis of the phylogeny reveals that the orthologue duck billed platypus, a semi-aquatic mammal outgroups aquatic and reptiles while showing 100 bootstrap value i,e, 100% similarty indicating presence of a common ancestor. In general, Rabbit *Oryctolagus cuniculus* is grouped with rodents as their similarties are a result of convergent evolution and share common lineage with rat *Rattus norvegicus*, mouse *Mus musculus* and guinea pig *Cavia porcellus*. Clade horses Equus caballus separates and outgroup among mammals with less significance. Mammals like pig *Sus scrofa*, sheep *Ovis aries* and cow *Bos tarus* are closely associated. In the constructed tree, two significant outgroups were seen, one group being includes the mammal african elephant *Loxodonta Africana* and the other being small eared galago *Otolemur garnetii* (primate) both of which are closely related and other group consisting of 13 lined ground squirrel *Spermophilus tridecemlineatus* (rodent) and dog *Canis familiar* (Canidae). These two outgroups signifies their similar functional importance which may include higher memory and sharp olfaction's. As shown in figure. 4a, condensed tree includes ancestral primates to modern human. Human exhibits a combined relationship with northern white cheeked gibbion *Nomascus leucogenys,* gorilla *Gorilla gorilla* and chimpanzee *Pan troglodytes* showing they derived from a common ancestor. White tufted ear marmoset *Callithrix jaccluus,* rhesus macaque *Macaca mulatta* and sumatran orangutan *Pongo abelli* are closely placed with high significance along with it, suggesting the descent exist. Furthermore, that this gene-protein were highly retained in fish to modern human pertaining to its functional importance.

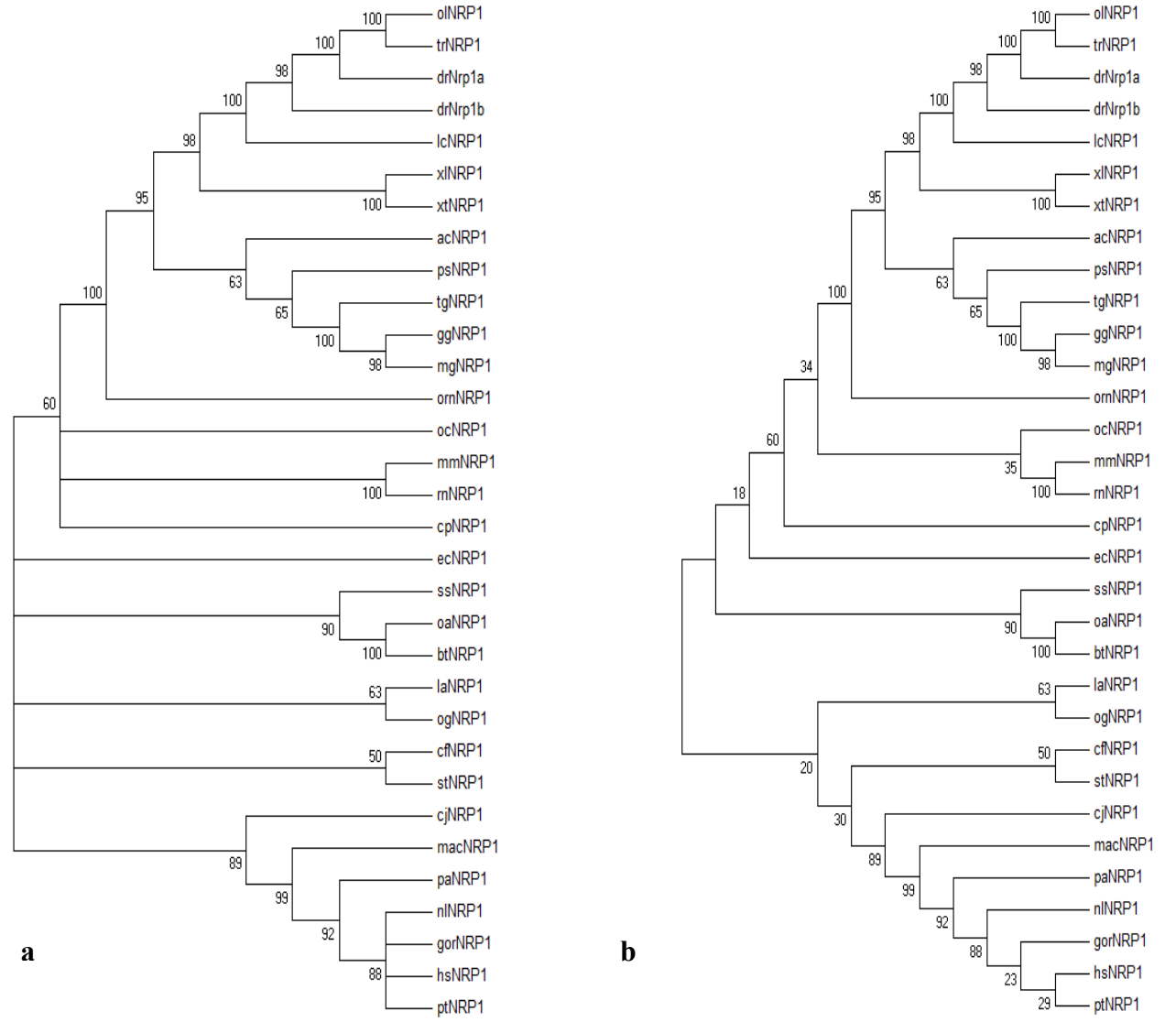



**Figure 4: Phylogenetics analysis of NRP 1.** Phylogenetics analysis of NRP1 with vertebrate sequence from each vertebrate group using Maximum parasimony algorithm. a) Condensed tree with cut off value 50 and b) Original tree with individual branch value. Bootstrap values are indicated on branches as a percentage of 1000 replicates.

**NRP1 b1 domain analysis**

Studies by Gu C [7] and Vander Kooi CW [10] have shown that b1 domain region is important for both SEMA3 and VEGF165 signaling. Analyses was carried out on NRP1 b1 region and noted that among all model animals, this region was highly conserved particularly at the specific ligand binding sites. These binding sites are named as L1, L2 and L3 pocket. The region specific amino acids of L1 are QYS/G/N, L2 region is K/EDT/S and L3 region being ISQ/KETK/RK-/KYY/F. While L1 showed variations from S298 to N298 and G298 in zebrafish *Danio rerio* drNRP1b and rat/mouse respectively, while in L2 varied from E319 to K319 and S321 to T321 only in zebrafish *Danio rerio* drNRP1a. A few more amino acids transition in L3 pocket was observed in frog. These pockets harbor the specific VEGF binding residues, namely Y297 in L1, D320 in L2 and S346,

T349, and Y353 in L3. The essential residues are known to be 'C-terminal Arginine binding pocket', across all species from amphibians to human and these amino acids shows 100% conservation, suggesting its crucial role in VEGF binding. In figure 5, b1 domain regions are represented in orange, while L1, L2 and L3 were highlighted in purple. Global blast analysis against NCBI showed that the L3 possesses a region ISKETKKKYY motif, which is unique to NRP1 and no VEGF binding protein analysed in the genomic databases of all the species other species thus making L3 region a unique binding pocket for $VEGF_{165}$ [24]. Conservation of this region indicates how NRP1 binding with $VEGF_{165}$ and SEMA signalling is crucial for developmental process. However, since there is no known secondary binding site, the basis for specificity in $VEGF_{165}$ binding remains unclear. In the b1 domain, FAV/VIIIC contains two conserved cysteins in most proteins, which link the extremities of the domain by a disulfide bond, region which may attribute to the proper folding.

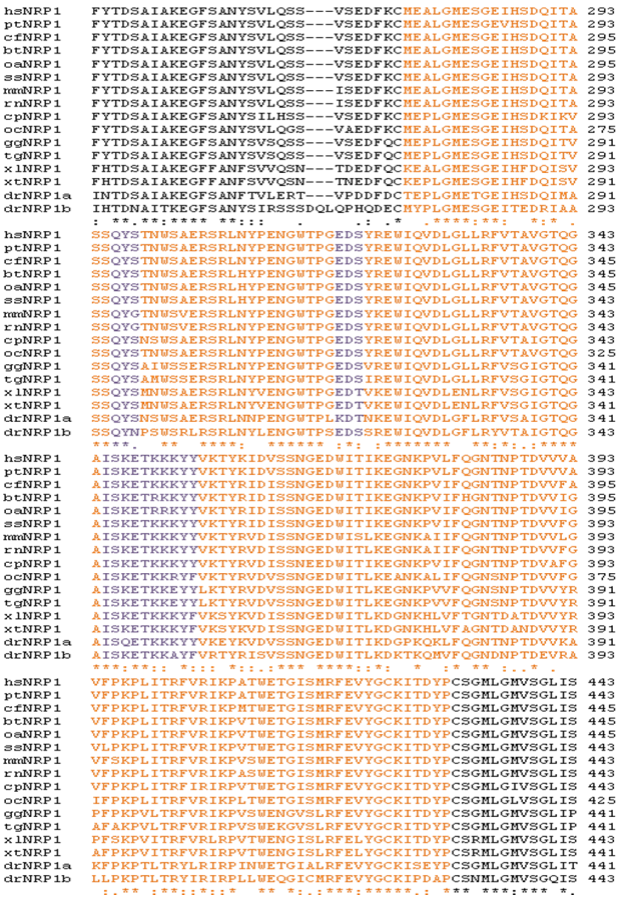



**Figure 5: MSA highlighting b1 domain region in NRP1.** Alignment of partial sequence of NRP1 from 15 species showing the 'b1 domain region sequence' (orange colour). Loops regions (L1, L2 and L3) that makes binding with VEGF ligand is highlighted in purple colour.

## 4. CONCLUSION

The NRP family is identified to play important roles in mediating efficient and effective signalling in VEGF, TGF-b, FGF and PDGF pathways in several cancer cell lines. Therefore targeting NRP may result in the disruption of both niche and the vasculature interaction with the tumor [25]. It is well known that b1 domain plays a vital role in both $VEGF_{165}$ and SEMA signaling. A close analysis of this region shows a high degree of among all animals. The knock-down studies in model system model have shown that the presence of NRP1 enhanced the binding of VEGF165 to KDR about 4-fold and increase endothelial cells chemotaxis towards VEGF, 2.5 fold greater than VEGF receptor 2, KDR alone [26]. The residues NRP1s Y297, W301, T316, D320, S346, T349, Y353 and W411 predicted to be in close contact with bound ligand VEGF are significantly conserved [27], [28]. To gain a better understanding of the mechanism by which NRP1 proteins signals in angiogenesis pathway, we presented here experimentally determined topological models for the unique family of NRP1 proteins showing conservation and followed by structure similarity analysis of human *Homo sapiens* and zebrafish *Danio rerio* NRP1-b1 domain revealing a both may function similar *in-vitro* system with similar structure that plays an important role in regulating angiogenesis conformation and function. Our phylogenetic analysis reveals billion years ancient common ancestral origin for NRP1 with evolutionary divergence and high rate of adaptation. In these various species analysis, lower the mutation rate higher the significance of receptor and its functional importance in survival. However, to date, experimental evidence does not exist to identify which portion or end of the b1 domain is involved in axon guidance cue and angiogenic factor binding [29]. In conclusion, NRP functions as a growth factor coreceptor in several signalling pathway implicating in cancer progression and metastasis [30]. Targeting NRP may address current issues in cancer biology such as cancer relapse and metastasis and improve patient prognosis [31], [32]. Therefore, NRP appears to be a novel and promising therapeutic target in the treatment of cancer.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

## HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

## CONSENT FOR PUBLICATION

Not applicable.

## AVAILABILITY OF DATA AND MATERIALS

The authors confirm that the data supporting the findings of this research are available within the article.

## FUNDING

None

**ACKNOWLEDGEMENT**

The authors are extremely thankful to the funding provided by NCNSNT and UGC-BSR.

**CONFLICT OF INTEREST**

Authors have no Conflict of Interest.

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