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In Silico Analysis of Foxo3 Gene from African Bush Elephant (*Loxodonta africana*)

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ABSTRACT: The *Foxo3* belongs to the O subclass of the forkhead family. *Foxo3* is a tumour suppressor, a master regulator of transcription of many genes, including those controlling lifespan extensions. *Foxo3* sequence of *Loxodonta africana* was analyzed in order to elucidate its potential functional role of its coding protein by *in silico* methods. Results showed that the *Foxo3* encoded a protein with 672 amino acid residues. The predicted protein with a forkhead domain was not a secretory protein, and was located in the nucleus. Foxo3 protein had high homology with that of other species. This study might provide a basis for future studies on the *Foxo3* gene of *Loxodonta africana* and functions of its encoded protein.

KEYWORDS: Loxodonta africana; In silico; Longevity; FOXO3

I. INTRODUCTION

The forkhead box (Fox) family of transcription factors originated in unicellular eukaryotes, and has evolved over time through multiple duplication events, and occasionally through gene loss, to members in mammals [1]. Fox genes have evolved to attain a specialized function in many crucial biological processes. *FOXO1*, *FOXO3*, *FOXO4* and *FOXO* come under O subclass of the forkhead family of transcription factors. They are characterized by a discrete ‘‘forkhead’’ domain and a transactivation domain, from the N-terminus to the C-terminus [2]. The forkhead domain amino acid motif helps in DNA binding and the trans-activation domain contains two conserved regions. The participation of trans-activation domain in DNA-binding activity has been demonstrated [3]. *FOXO3* regulates the transcription of many genes which sequentially mediate various cellular processes including DNA repair, glucose metabolism, detoxification of reactive oxygen species (ROS), cell-cycle arrest and cell death [4,5].

The living elephants (African and Asian) are the last survivors of a once highly successful mammalian order, the Proboscidea [6]. The elephants are included in the most prehistoric placental mammals and they belong to the placental super clade Afrotheria. *Loxodonta Africana*, the African bush elephant, has one of the longest life spans among mammals and no extensive studies have been conducted to unravel the molecular basis of longevity of this species. In the present study, *Foxo3* sequence of *Loxodonta africana* and its encoded protein was analyzed using *in silico* techniques. These results would provide a basis for further researches on *Foxo3* gene of *Loxodonta africana* and predicted functions of its encoded protein will aid the studies in lifespan research.

II. MATERIALS AND METHODS

Sequence analysis of *Foxo3*:

The forkhead box (Fox) O3 *Foxo3* sequence of *Loxodonta africana* (XM_003404305.2) was obtained from NCBI database (www.ncbi.nlm.nih.gov).The protein sequence was retrieved in FASTA format. The physiochemical properties of the selected sequence were analysed using Protparam tool (http://web.expasy.org/protparam/), which computes various physical and chemical parameters for a given protein sequence. Secondary structure predictions of the protein was done with SOPMA server [7] (https://npsa-prabi.ibcp.fr/) and PSIPRED (Predict Secondary Structure) (http://bioinf.cs.ucl.ac.uk/psipred/).

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Three dimensional structure generation and validation:

The three dimensional structure of *FOXO3* of *Loxodonta africana* was predicted by using Phyre[2] (Protein Homology/analogY Recognition Engine V 2.0). It is one of the most reliable structure prediction software's. The generated PDB format of the structure was viewed by using RASMOL visualization tool. Secondary structures like α-helix, β-sheets, random coils and extended strands were analysed using RasMol. The structure validations of the selected proteins were carried out using Ramachandran plot .The stereochemical analysis of the generated structure was analyzed by SOPMA on NPS@server (http://pbil.ibcp.fr/htm/index.php) [8] and PSIPRED (http://bioinf.cs.ucl.ac.uk/psipred/**)**.The transmembrane helices and topology of the Foxo3 were predicted by TMHMM software on ExPASy Proteomics Server (http://www.expasy.ch /tools). The signal peptide was predicted by SignalP (http://ww w.cbs. dtu. dk/services/SignalP/). The conserved domain was predicted by blastp and Conserved Domain Database (http://www.ncbi. nlm.nih.gov/ cdd) [9]. The subcellular localization of Foxo3 was predicted by PSORT Ⅱ (http://psort.nibb.ac.jp/) and SubLoc v1.0 (http:// www.bioinfo.tsinghua.edu.cn/).The multiple sequence alignment and construction of Neighbor-Joining phylogenetic tree was executed by Mega 6.0.

III. RESULTS

Sequence analysis of *Foxo3* of *Loxodonta africana*:

Sequence Analysis of *Foxo3* gene of *Loxodonta africana* was done in the NCBI BLAST(Fig1). The open reading frame predicted by ORF finder is shown in Fig 2.

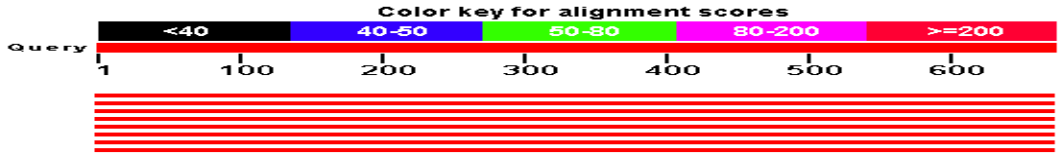

Fig.1. Result of blast *Foxo3* in *Loxodonta africana* genome database

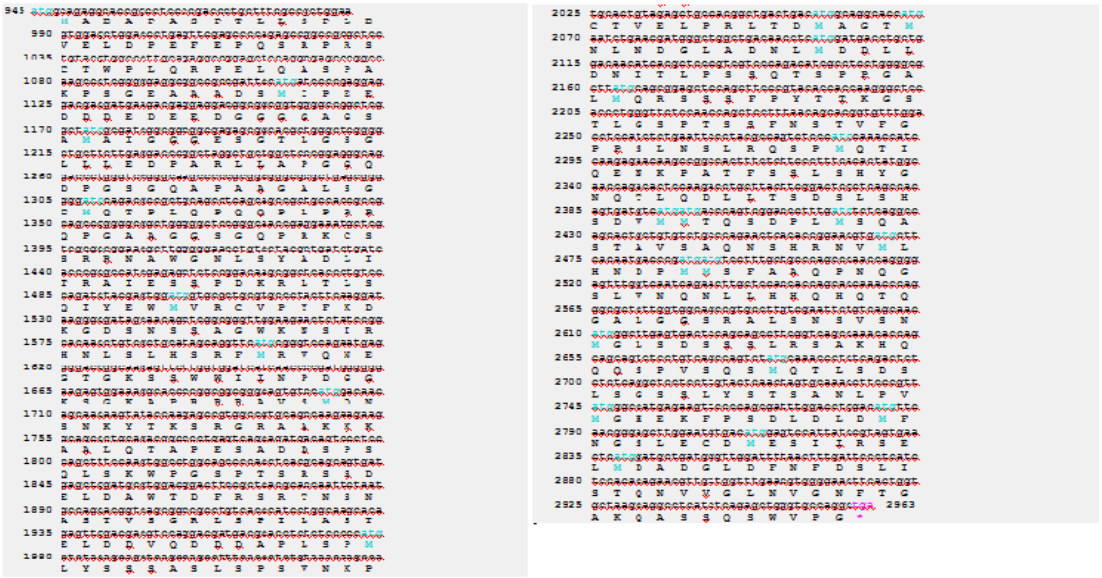

Fig. 2. The open reading frame predicted by ORF finder

The primary structure description of predicted *Foxo3* protein:

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The retrieved FASTA sequence of the *Foxo3* protein sequence was subjected to ProtParam analysis to analyse physiochemical properties. The analysis result of ProtParam is shown in Table 1 and atomic composition of the protein is given below.

Number of amino acids	672
Molecular weight	71355.7
Theoretical pI	4.93
Total number of negatively charged residues (Asp + Glu)	72
Total number of positively charged residues (Arg + Lys)	50
Extinction coefficients (M-1 cm-1) at 280 nm measured in water	61670 assuming all pairs of Cys residues form cystines. 61420 assuming all Cys residues are reduced
Estimated half-life	30 hours (mammalian reticulocytes, *in vitro*).
Instability index (II)	64.56
Aliphatic index	63.81
Grand average of hydropathicity (GRAVY)	-0.589

Table1. ProtParam results

Atomic composition:
Carbon C - 3037
Hydrogen H - 4811
Nitrogen N - 885
Oxygen O - 1042
Sulfur S - 30

The secondary structure of predicted Foxo3 protein:

According to the result analyzed by SOPMA, the secondary structure prediction tool (Fig 3), Foxo3 possessed 27.83% of α-helics,11.90% of extended strand, 6.70% of β-strands and 53.57% of random coils. The secondary structure was also predicted using another tool PSPIRED (Fig 4). Foxo3 protein was not a trans-membrane protein from the result of TMHMM (Fig 5). From the prediction of SignalP it was not a secretory protein and had no signal peptide. The predicted results of BLASTp and CDD suggested a conserved domain similar with the forkhead domain in the protein, which was a DNA binding region. These results designate the predicted *Foxo3* to be a transcription factor.

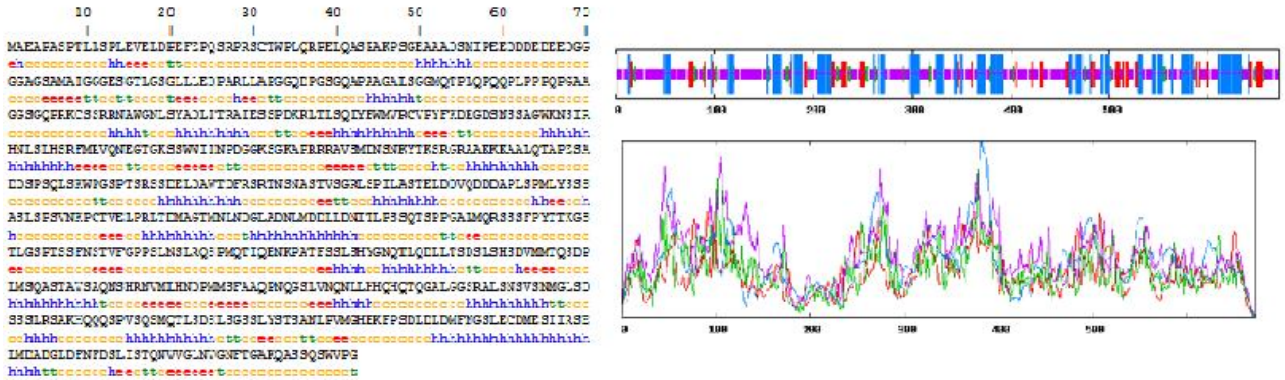

Fig.3. The secondary structure of predicted Foxo3 protein predicted by SOPMA

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Fig .4. The secondary structure of predicted Foxo3 protein predicted by PSIPRED

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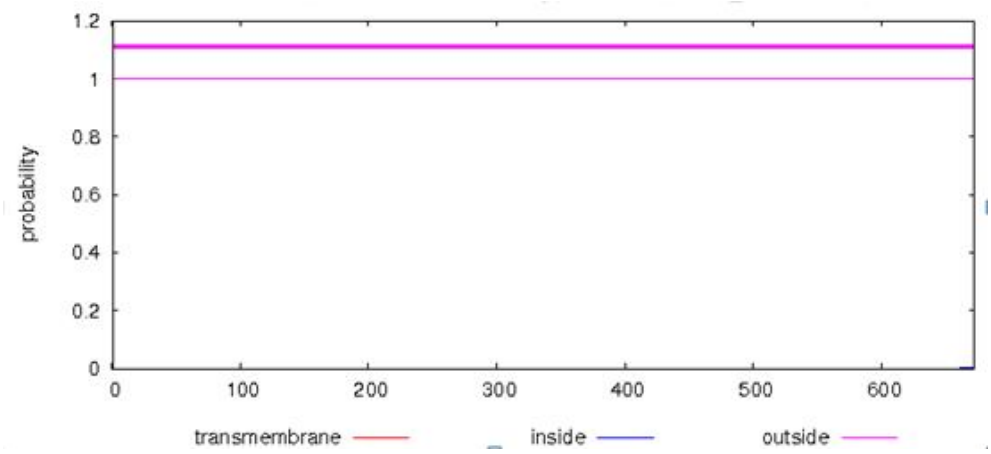

Fig.5. Transmembrane helices of predicted Foxo3 protein predicted by TMHMM

3D Structure Generation & Validation:

Three dimensional structure of *FOXO3* was predicted using Phyre[2]- webportal for protein modeling. The predicted structure is shown in Fig 6.

Fig.6. *FOXO3* predicted structure in Rasmol viewer

The structure has 882 atoms, 900 bonds, 5 helices, 13 turns and 3 strands. It also constitutes 55 H-bonds.
The result was analysed using Ramachandran plot which showed that 87.2 % residues are in the allowed region. The results are shown in Fig 7 and Table 2.

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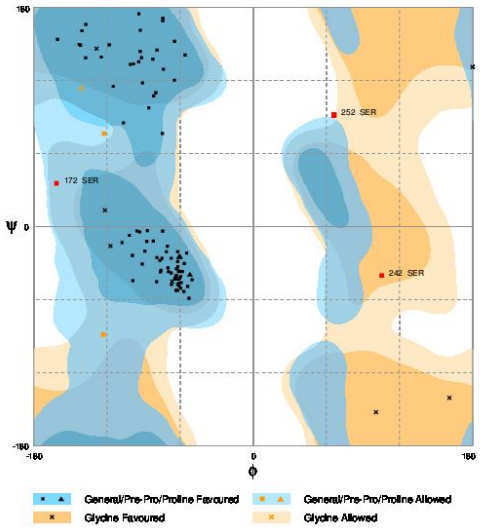

Fig.7. Ramachandran of of predicted structure of Foxo3 protein

Plot Details	Foxo3
Residue in most favoured region (%)	93.9%
Residues in additional allowed regions (%)	3.0%
Residues in disallowed regions (%)	3.0 %

Table 2. Ramachandran plot details

Subcellular localization of predicted Foxo3 protein:

The results of PSORT Ⅱ showed that the predicted Foxo3 protein was located in nucleus. The expected accuracy was 73.9%.

Molecular phylogenetic analysis of Foxo3 protein:

The BLASTp result of predicted Foxo3 protein indicated the high homology of *Foxo3* protein between different species. The phylogenetic tree showed in Fig 8. The evolutionary history was inferred using the Neighbor-Joining method.

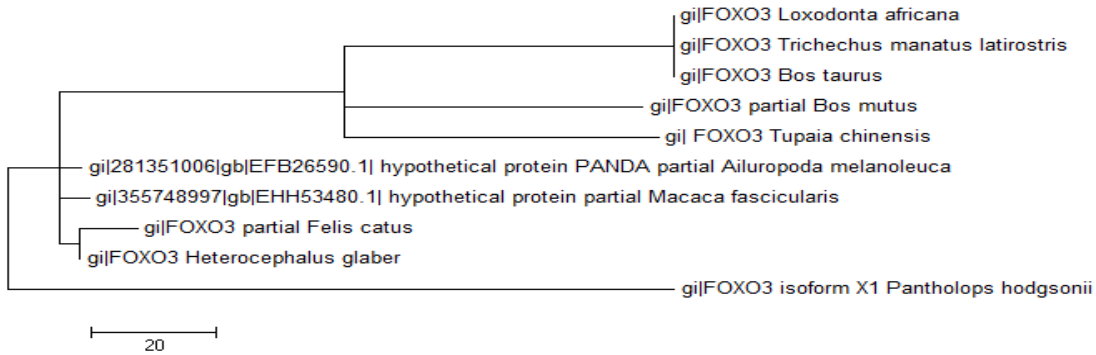

Fig.8. Phylogenetic tree of Foxo3 protein of different species.

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IV. CONCLUSION

In silico cloning and bioinformatics analysis of *Foxo3* and its encoded protein suggested that *Foxo3* had 3,912 base pairs. The ORF finder predicted that the *Foxo3* of *Loxodonta africana* encoded a protein with 672 amino acid residues. The predicted protein was not a secretory protein, and it was located in nucleus. Transmembrane helices of predicted Foxo3 protein were predicted by TMHMM. The subcellular localization of a protein usually has very close relationship with protein function. Foxo3 was a transcription factor with a DNA binding region, so it probably was a nuclear protein. This was in accordance with the predicted result of PSORTII. The forkhead domain acted as a DNA binding site. The predicted protein had a conserved domain which had high homology to forkhead superfamily. Members of the class O of forkhead box transcription factors (FoxO) have important roles in metabolism, cellular proliferation, stress tolerance and probably lifespan. Predicted sequence had close relationship with the function of *Foxo3* as a transcription factor which regulates the transcription of many genes including longevity related genes. The three-dimensional structural features of the protein, generated in this study, were highly useful in better understanding the activity of the protein.

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