

Modeling and Analysis of c-Ski Subunit that Interacts with R-Smads in TGF- β Signaling Pathway

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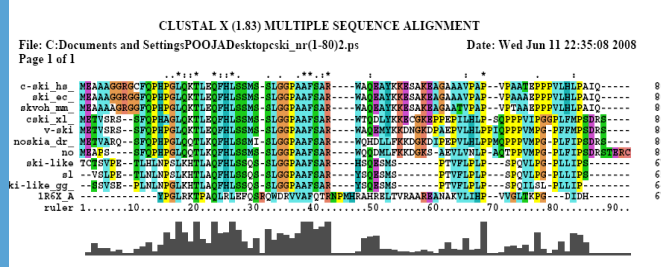


Introduction

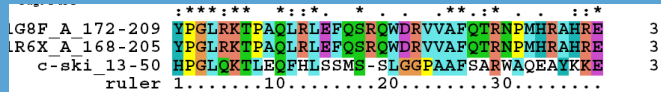
- The Smad proteins are considered to be tumor suppressors as they play central role in mediating the growth inhibitory response of TGF- β .
- Inactivation of the Smad proteins either by deletion or by mutations were shown to result in malignant progression.
- c-Ski and SnoN, are transcriptional co-repressors that inhibit TGF- β signaling through their interaction with Smad protein which results in disruption of an active heteromeric Smad complex and displacement of the transcriptional co-activators, p300/CBP, and therefore may be responsible for the transforming activity.
- The c-Ski was identified as the cellular counterpart of v-ski oncogene which was first isolated from retroviruses in Sloan Kettering Institute.
- It was been shown by various studies that c-Ski specifically associates with TGF- β /activin regulated Smads (Smad2/3) and co-Smad (Smad4) and doesn't interact with the BMP regulated Smads (Smad1/5/8) or I-Smads (Smad6/7).
- Crystal structure of c-Ski (residues 219-312) binding with Smad4 is known however the region that binds to Smad3/2 is not known which we modeled using two remote homologous templates.
- c-Ski is known to bind simultaneously to Smad4 and R-Smads through Smad interaction domains forming Smad2/3-Smad4 hetero-oligomer and c-Ski homo-oligomer.
- The model of c-ski provides a structural framework for further investigation of Ski-Smad interactions and may help design inhibitors to regulate TGF- β signaling.

Methods

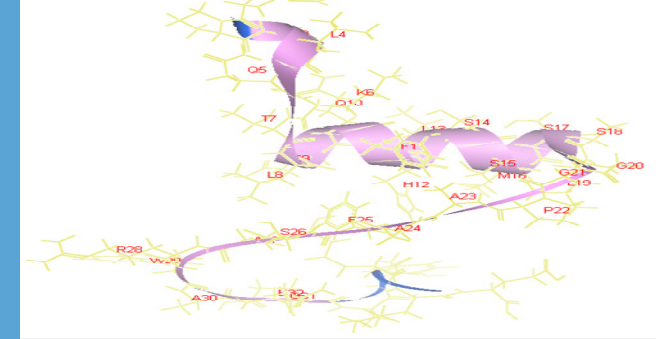
- Residues 17-45 of c-ski are known to interact with Smad3 therefore the sequence region of 1-80 residues of c-ski was extracted from NCBI database. Secondary structure prediction was done using JPRED and PSI-PRED.
- Homologues of c-ski (1-80) were searched in refseq database of protein sequences at NCBI using PSI-Blast.
- Blast and several publicly available fold recognition and remote homologous sequence detection servers were run against PDB for c-ski (1-80) to find a suitable template for homology modeling.
- Multiple sequence alignments were generated for c-ski, template and the distant homologous sequences found in refseq database along with the identified templates in PDB (1g8f & 1r6xA) using Clustal X.
- Model building was performed using MODELLER (Discovery studio v2.0), SWISS-MODEL, CPH, 3DJigsaw and InsightII modeling software using 1g8fA as template.
- Each of the 15 models generated were evaluated using structure evaluation methods PROCHECK, VERIFY3D, WHATCHECK, PROVE and PROSAll verification servers.



Alignment of c-ski with templates



Selected Model of c-ski subunit that interact with Smad2/3

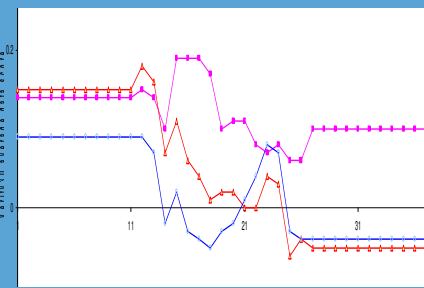
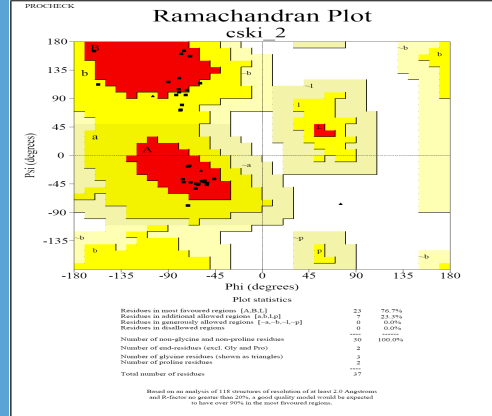


Percentage similarity and identity of c-ski (1-80) with respect to templates

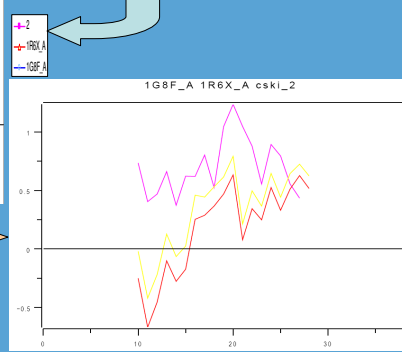
	Identity	Similarity	Bits Score	E-value
1G8F_A	31	43	26.2	2.8
1R6X_A	31	43	25	5.9

Model Evaluation

Procheck (Stereochemical quality) of selected Model



Verify3D curves of best selected model. This score measures the compatibility of model with its sequence using a scoring function, if more than 80% of the residues has a score of >0.2 then the protein structure is considered of high quality



PROSA II energy graphs of selected model (magenta) and template (red and yellow). PROSA captures the average properties of native globular proteins in terms of atom pair, and protein-solvent interactions which shows consistent with a reliable conformation based on its similarity with that of the template.

Conclusion

With the knowledge of biochemical data we have identified remote homologous templates of c-Ski Smad2/3 binding region and performed homology modeling of this region of the structure. The structural region of c-ski that binds to r-Smad – Smad4 complex can be used to design specific small molecular inhibitors that antagonize c-Ski binding which may possibly carry anti-cancer properties. Our future plan is to design such small molecule through virtual screening.

References

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