Developing a multidisciplinary strategy to interpret the impact of missense mutations in XPA on NER activity and cisplatin sensitivity

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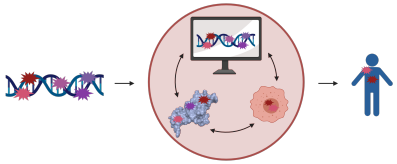
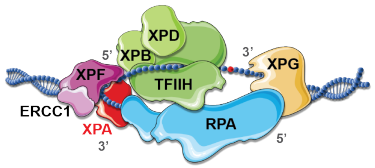
1. Current efforts to predict the impact of VUS can be improved.



- Predictive, correlative data is insufficient for robust evaluation of VUS.
- Evidence-based validation is required to identify variants with clinical utility.

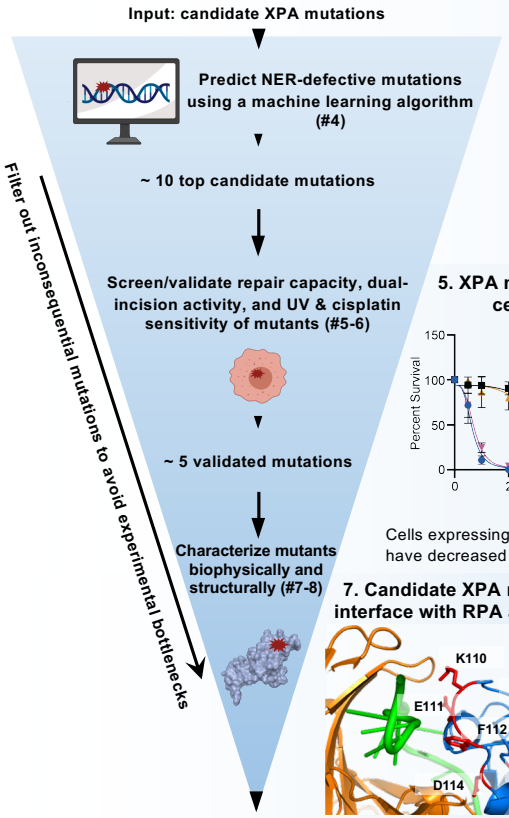
2. Nucleotide Excision Repair (NER) genes are mutated in cancers.



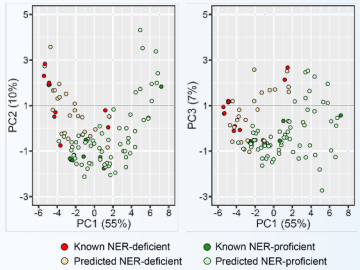
Gene	Protein	Number of missense mutations (PanCanAtlas)
ERCC1	ERCC1	58
ERCC2	XPD	104
ERCC3	XPB	94
ERCC4	XPF	117
ERCC5	XPG	196
RAD23B	RAD23B	53
RPA1	RPA70	55
RPA2	RPA32	34
RPA3	RPA14	12
XPA	XPA	30
XPC	XPC	92

- NER removes bulky DNA lesions formed by UV and cisplatin.
- Mutations in *ERCC2* correlate with improved patient survival after cisplatin treatment.
- The impact of most NER mutations is unknown.

3. Pipeline to identify deleterious XPA mutants.

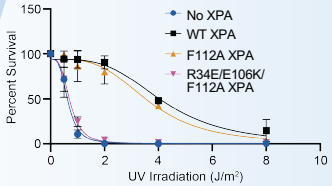


4. Machine learning algorithm predicts impact of XPA mutants.



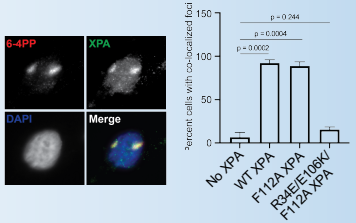
- 108 input mutants:
  - 11 known NER-defective
  - 8 known NER-competent
  - 89 unknown from literature and tumor genome databases
- 20 predictive scores for each from web-based tools
- Leave-one-out cross validation performed

5. XPA mutations impact cell survival.



Cells expressing NER-defective XPA mutants have decreased survival after UV irradiation.

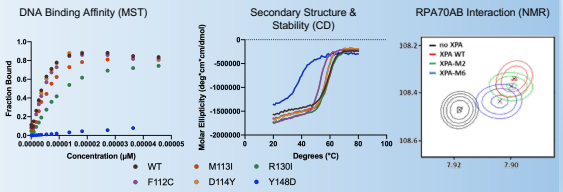
6. XPA mutants impact localization to UV lesions.



Cells expressing NER-defective XPA mutants have decreased co-localization with UV lesions.

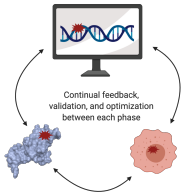
7. Candidate XPA residues interface with RPA and DNA.

8. XPA mutants can be characterized biophysically and structurally to determine the mechanism of dysfunction.



Missense mutations in XPA can impact DNA binding affinity, interaction with RPA, protein stability, and dual-incision activity.

9. Summary and Future Directions



Current strategies to predict and interpret the impact of VUS can be improved by incorporating functional validation.

Future directions include:

1) Experimental validation of the machine learning algorithm;
2) Cell-based, biophysical, and structural characterization of selected candidate mutations;
3) Expansion of the pipeline to other core NER proteins.

10. Acknowledgements

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11. References

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