Nucleotide excision repair deficiency is a hypoxia regulated, targetable therapeutic vulnerability in clear cell renal cell carcinoma.

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Abstract: Background Due to a demonstrated lack of DNA repair deficiencies, clear cell renal cell carcinoma (ccRCC) has not benefitted from targeted synthetic lethality-based therapies. We investigated whether nucleotide excision repair (NER) deficiency is present in an identifiable subset of ccRCC cases that would render those tumors sensitive to therapy targeting this specific DNA repair pathway aberration.

Results: ccRCC cell lines showed various degrees of NER deficiency by functional assays under normoxic conditions. In hypoxia, NER activity was reactivated. Some of these cell lines also showed increased sensitivity to irofulven. This sensitivity was also correlated with the expression of prostaglandin reductase 1 (PTGR1), an enzyme required for the activation of irofulven. Next generation sequencing data of the cell lines indicated the presence of NER deficiency associated mutational signatures. The same signature as well as significant expression levels of PTGR1 was detected in a significant subset of ccRCC patients.

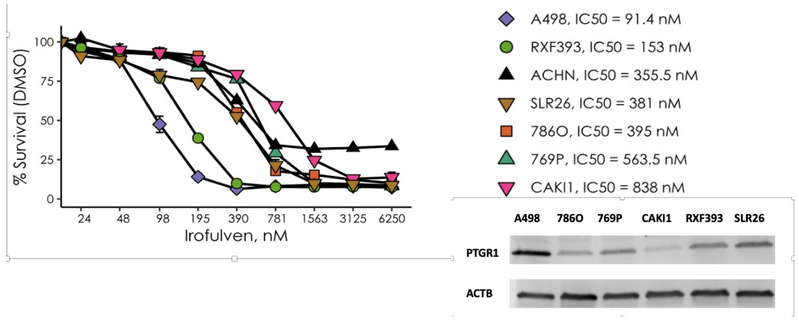
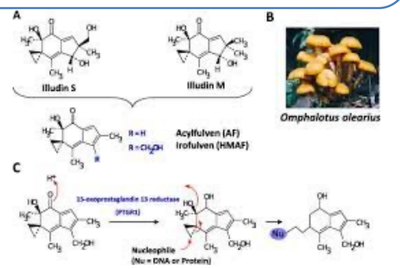


Figure 1. Kidney cancer cell lines show various degrees of sensitivity to irofulven, an experimental therapeutic agent with high specificity to transcription coupled repair deficient cells. In vitro cell viability assays indicating some cell lines having an effective IC50 around 100 nM., which is in the clinically validated tolerable concentration range in phase 2 clinical trials. PTGR1 expression an enzyme required for the activation of irofulven, was monitored by western blotting.



Irofulven is an experimental cancer therapy agent in phase 2 clinical trials with a favorable toxicity profile. It is highly specific in cells with transcription coupled repair (TCR) deficiency and requires activation by prostaglandin reductase 1 (PTGR1)

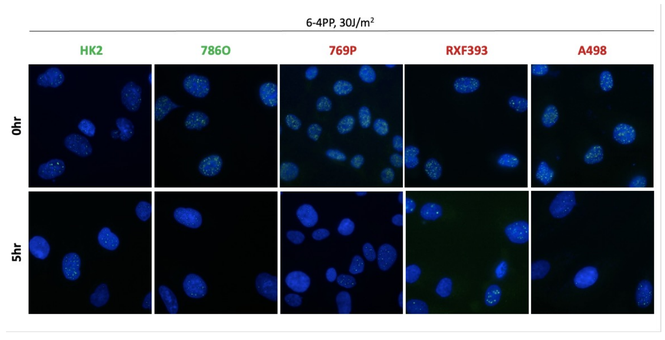


Figure 2. Kidney cancer cell lines show various degrees of NER deficiency as detected by the removal of UV induced 6-4PP photoproducts: Cells were irradiated with UV at the indicated those and UV induced 6-4PP products were visualized by detected by antibody staining.

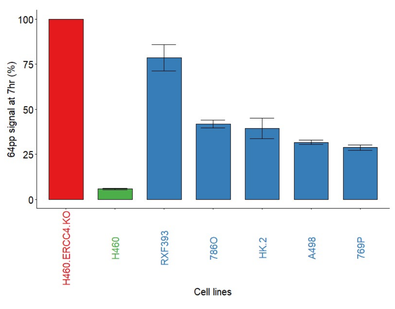


Figure 3. Quantification of the NER deficiency described in Figure 2. The percentage of 6-4PP photoproducts not removed after 7 hours. Cell lines showed varying levels NER deficiency. The H460 cell line served NER proficient control (completely removing the photoproduct, green bar) and the engineered ERCC4 deleted version of the same cell line served as NER deficient control (red bar).

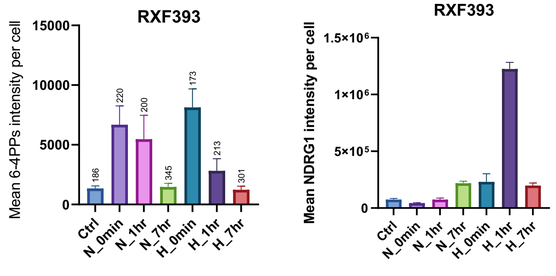


Figure 4. Hypoxia (1% oxygen) reactivates NER activity in the RXF393 cell line. NER deficiency was quantified as described as in figure 2 in the RXF393 cell line under normoxic and hypoxic (1%) conditions. NDRG1 expression levels were also monitored as a control.

Methods: We used functional assays that detect UV-induced 6-4 pyrimidine-pyrimidone photoproducts to quantify NER deficiency in ccRCC cell lines. We also measured sensitivity to irofulven, an experimental cancer therapeutic agent that specifically targets cells with inactivated transcription-coupled nucleotide excision repair (TC-NER). In order to detect NER deficiency in clinical biopsies, we assessed whole exome sequencing data for the presence of an NER deficiency associated mutational signature previously identified in ERCC2 mutant bladder cancer.

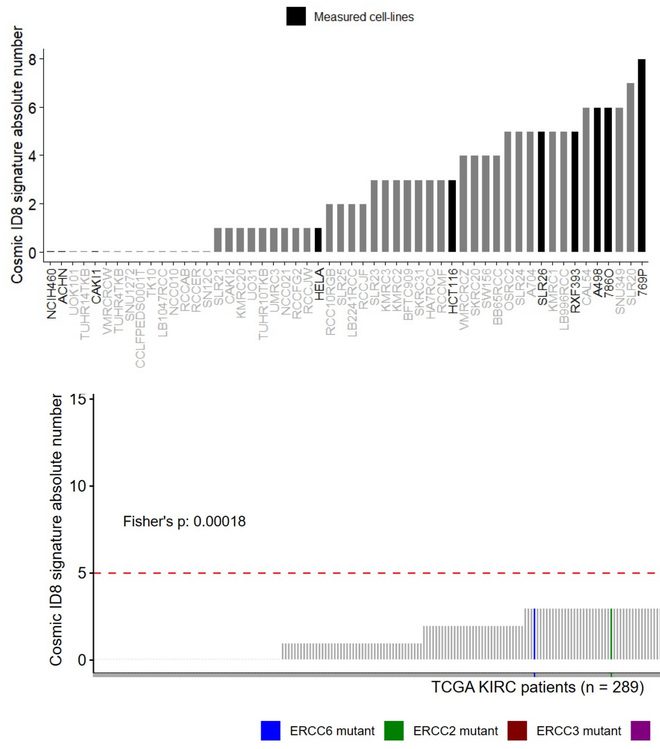


Figure 5. ccRCC cell lines with functional NER deficiency also display an NER deficiency associated mutational signature. We analyzed the whole exome sequencing data of ccRCC cell lines. We previously established that more than five ID8 deletions detected in WES analysis indicates the likely presence of NER deficiency in bladder cancer (Borcsok et al.). Using the same criteria we found that several of the cell lines displaying functional NER deficiency also harbored the same mutational signature.

Figure 5. A subset of clear cell carcinoma clinical cases display a mutational signature of NER deficiency and PTGR1 expression. We analyzed the whole exome sequencing data of 289 kidney cancer cases in TCGA. We previously established that more than five ID8 deletions detected in WES analysis indicates the likely presence of NER deficiency in bladder cancer (Borcsok et al.). 43 out 289 ccRCC cases (~15%) had ID8 deletion numbers consistent with NER deficiency. 36 of the 43 cases with ID8 deletion number higher than five had significant PTGR1 expression as well. Considering these criteria 36 of the 289 TCGA cases (~12%) indicated the presence of both NER deficiency and significant PTGR1 expression levels thus defining the proportion of clear cell renal carcinoma cases that may respond to irofulven therapy.

Translational relevance:

1) **10% of ccRCC cases may harbor NER deficiency that is therapeutically targetable by irofulven or platinum**
2) **In a further subset of ccRCC cases NER deficiency can be induced either by altering oxygen concentration (carbogen treatment) or manipulating other hypoxia regulated factors, thus rendering those cases either irofulven or platinum sensitive.**

Conclusions: ccRCC cell line based analysis showed that NER deficiency is present in this cancer type. Approximately 10% of ccRCC patients in the TCGA cohort showed mutational signatures consistent with ERCC2 inactivation associated NER deficiency and also substantial levels of PTGR1 expression. These patients may be responsive to irofulven, a previously abandoned anticancer agent that has minimal activity in NER-proficient cells.

We also found that NER deficiency is induced by normoxia in ccRCC cell lines. We are currently exploring whether this is a general phenomenon if ccRCC cell lines and also aiming to identify the actual regulatory mechanism. Oxygen levels can be increased in cancer tissue by e.g. carbogen inhalation and thus it may be possible to induce NER deficiency in the therapeutic setting.

Borcsok et al: ***Identification of a Synthetic Lethal Relationship between Nucleotide Excision Repair Deficiency and Irofulven Sensitivity in Urothelial Cancer. Clin Cancer Res 2021 Apr 1;27(7):2011-2022.***