Exoribonuclease XRN1 is a Therapeutic Vulnerability in Tumors with Intrinsically Elevated Type I Interferon Signaling

Maureen M. Lynes[1], Sophie A. Shen[1], Sunaina P. Nayak[1], Brian A. Sparling [1], Anugraha Raman[1], Jie Wu[1], Scott Ribich[1,2], Stephen J. Blakemore[1], and Serena J. Silver[1].

[1]Accent Therapeutics, Lexington, MA, USA [2]Current Address: Blueprint Medicines, Cambridge, Massachusetts

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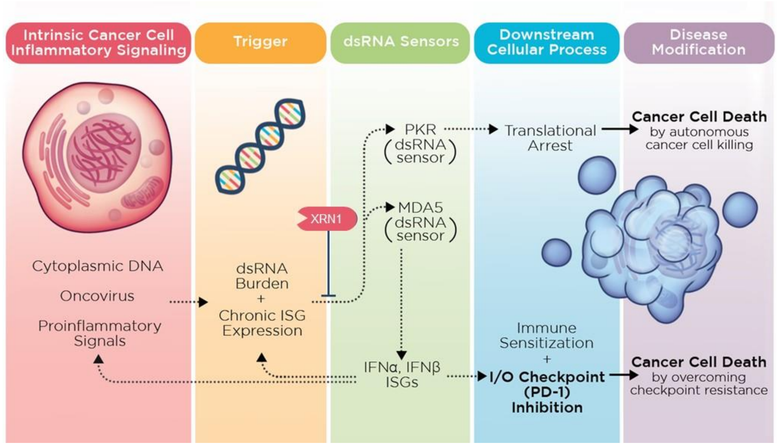
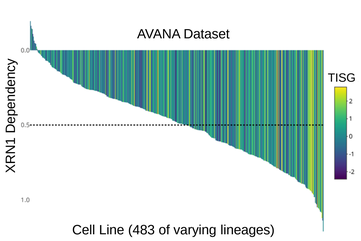
Exoribonuclease XRN1 is an Exciting Oncology Target and Selective Vulnerability in Tumors with Elevated Interferon

- 5′→3′ exoribonuclease 1 (XRN1) degrades single stranded mRNA from the 5′→3′ direction and is important for endogenous cellular mRNA turnover[1]
- XRN1 can also degrade double-stranded RNA (dsRNA), and plays a role in innate immunity by preventing dsRNA activation of the cytosolic sensors MDA5 and pPKR[2]
- Analysis of publicly available CRISPR screens has identified XRN1 as a potential synthetic lethality target in tumor cells with intrinsic elevation of a Type I Interferon Stimulated Gene (TISG) signature
- Knockout of XRN1 in TISG high cells results in cell death and downstream activation of the MDA5 and PKR innate immune pathways
- Interferon β (IFNβ) stimulation of TISG low cells sensitizes those cells to XRN1 loss
- Based on these results, and consistent with recently published literature[3,4,5], XRN1 is a compelling target for monotherapy in TISG high tumors, and in combination with immuno-oncology therapeutics

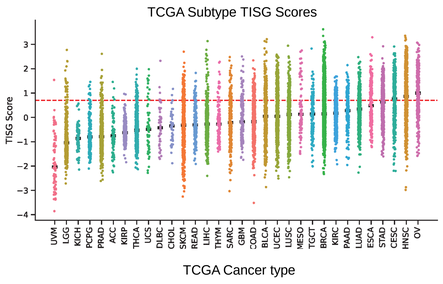


A Type I Interferon Gene Signature Predicts Sensitivity to XRN1 Inhibition

Cell Lines With Elevated Type I IFN Signaling (TISG score) are Dependent on XRN1



TISG-High Score Exist Across Multiple Cancer Subtypes in the TCGA

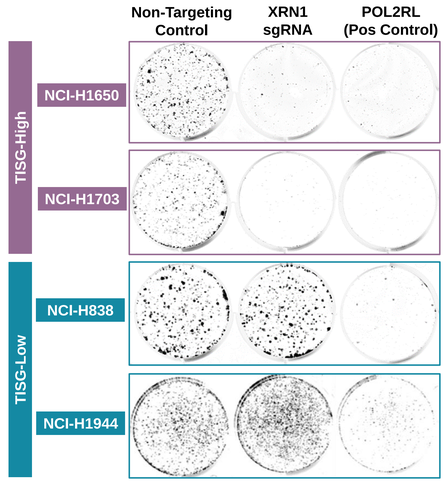


- Accent TISG score is determined by expression of a custom 26 gene subset of type I interferon–stimulated genes that predict XRN1 dependence
- ~15-30% of primary TCGA tumors display elevated Type I Interferon signaling, with enrichment in HNSCC, ovarian, cervical, lung and breast cancer

References

1. Jinek *et al*, Mol Cell, 2011
2. Burgess *et al*, Cell Host Microbe, 2015
3. Zhou *et al*, bioRxviv, 2023 (preprint)
4. Ran *et al*, Cancer Research 2023
5. Hosseini *et al*, bioRxviv 2023 (preprint)

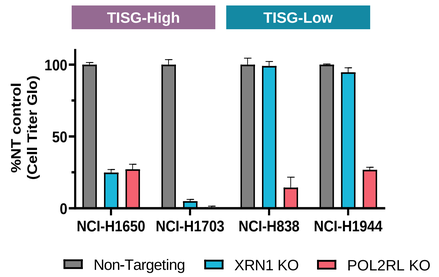
XRN1 KO Selectively Inhibits Colony Formation in TISG-High Cells



- TISG-high (NCI-H1650 and NCI-H1703) and TISG-low (NCI-H838 and NCI-H1944) cells were transduced with XRN1 sgRNA and subjected to colony formation assay for 10-14 days
- XRN1 KO dramatically decreases colony formation in cell lines with endogenously high type I IFN signaling
- XRN1 KO does not affect colony formation in the predicted insensitive, TISG-low cell lines

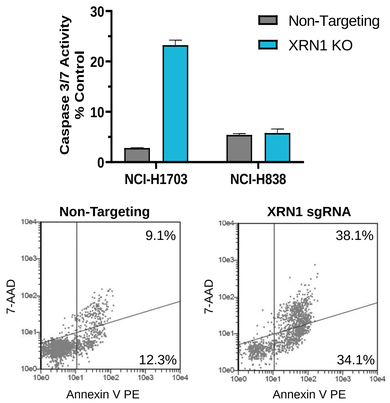
XRN1 KO Inhibits Proliferation of TISG-High Cells; TISG-Low Cells Tolerate XRN1 Loss

- TISG-high and TISG-low cells were transduced with XRN1 or POL2RL (positive control) sgRNA for 12 days, and anti-proliferative activity was measured using the Cell Titer Glo assay
- XRN1 KO leads to robust anti-proliferative activity in TISG-high cells; no impact on proliferation of the predicted insensitive TISG-low cells was observed despite XRN1 KO

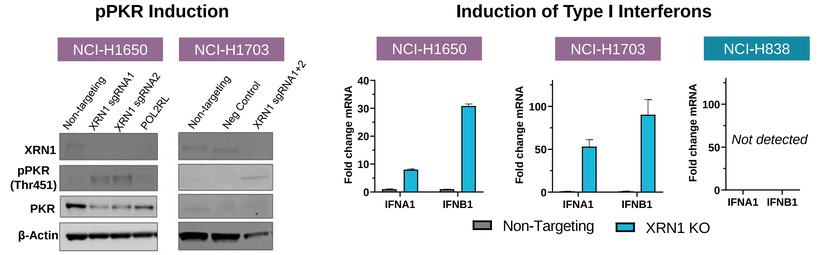


XRN1 Knockout Induces Apoptosis in TISG-High Cells

- NCI-H1703, NCI-H838, and NCI-H1650 cells were transduced with XRN1 sgRNA; apoptosis was measured using Caspase 3/7 glo (7 days post-transduction) or Annexin V /7AAD staining (10 days)
- XRN1 KO induces Caspase 3/7 activity robustly in the TISG-high NCI-H1703 cell line but not the NCI-H838 line
- 72.2% of NCI-H1650 cells are Annexin V positive as assessed by flow cytometry at day 7, compared to 21.4% of NT control

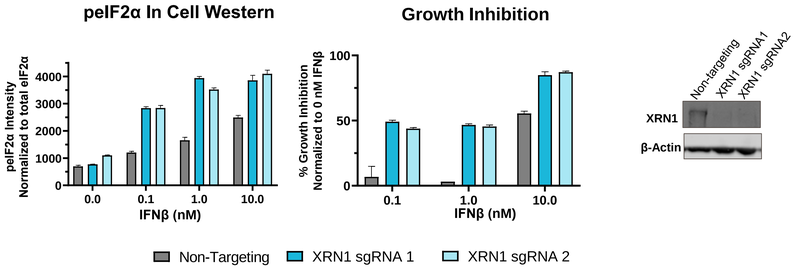


XRN1 KO Activates PKR and MDA5 Innate Immune Pathways



- TISG-high and TISG-low cells were transduced with XRN1 sgRNA for 7 days, following which protein and RNA was extracted for western blotting and qPCR
- XRN1 KO leads to an increase in pPKR levels and upregulation of type I interferons in XRN1 dependent TISG-high cells only; no pPKR or IFN induction is detectable in TISG-low cells

Type I Interferon Stimulation Sensitizes TISG Low Cells to XRN1 Loss



- Induction of peIF2α is observed in XRN1 KO but not WT TISG-low cells upon IFNβ stimulation (96 hours); this corresponds with anti-proliferative effects and is consistent with activation of the MDA5 pathway
- Together these results suggest that suggesting that XRN1 KO sensitizes otherwise insensitive cells to activation of the dsRNA response in the presence of elevated exogenous interferon, providing a rationale for combination of XRN1 loss and checkpoint inhibitors

Conclusions

- Accent has developed a gene score (TISG) representing a subset of interferon-stimulated genes that predicts dependency on XRN1
- Knockout of XRN1 using CRISPR validates the selective dependency of TISG-high cell lines on XRN1; TISG-low cell lines tolerate XRN1 loss with no anti-proliferative effects
- Loss of XRN1 activates the PKR and MDA5 innate immune sensors, consistent with accumulation of dsRNA downstream of XRN1 loss
- IFNβ stimulation of a TISG low cell line sensitizes it to XRN1 loss, providing rationale for combining XRN1 loss with immunotherapy
- XRN1 is a promising target with mono-therapy potential in TISG high tumors, and the potential to enhance the efficacy of checkpoint inhibitors through modulation of innate immune pathways

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