Differential expression of microRNA between standard conventional dose versus ablative dose radiation in colorectal cancer
Shushan Rana1, Cristina Espinosa2, Rebecca Ruhl1, Charles R Thomas Jr1,3, and Sudarshan Anand1,2,3
1Dept of Radiation Medicine, 2 Cell, Developmental & Cancer Biology, 3Knight Cancer Institute, Oregon Health & Science University

Abstract

- Biologic heterogeneity of cancer offers a spectrum of radiation responsiveness warranting radiation dose escalation for certain cancers.
- However, clinicopathologic propensity for extensive microscopic spread negates strategies to reduce treatment volumes thus inhibiting safe dose escalation.
- A major regulator of the ionizing radiation (IR) response is microRNA (miRNA) with the tumor and tumor microenvironment (TME)
- Critical function in cancer biology
- Regulate hundreds of genes in a gene network through differential sequence complementarily.
- In this context, we hypothesized the influence of radiation dose escalation on IR-mediated damage will be detectable through differential IR-induced miRNA expression patterns.

miRNAs exhibit differential expression with increasing doses or radiation in CRC

Fold change relative to control non-irradiated tumor | Red indicates increase miR; Green indicates decreased miR

Data processing via nSolver 3.0:
1) Background subtraction of negative controls
2) Counts normalized to GAPDH, Aact, Rpi19 housekeeping genes
3) Fold change calculated relative to 0 Gy

miRNA selection literature review:
1) miR in relation to CRC
2) miR in relation to radiation
3) Gray cells indicate minimal to no literature associated with miR
4) Blue cells indicate the currently studied lab miRNAs

Table 1. miRNA expression in CT-26 xenografts (as described earlier) post-radiation at 6 h

Figures

Figure 1. We utilized a murine implantation model of CT26 colorectal adenocarcinoma adenocarcinoma flank xenografts (syngeneic BALB/c) mice. Mice received either no irradiation, 2 Gy, 5 Gy, or 10 Gy single dose Co-137 flank irradiation administered through lead shielding. At 0 hour post-IR, tumors were harvested. We used the Nanosmart miRNA profiling platform and obtained absolute counts for 600 mouse miRs. Nanoshr software was used for data normalization and analysis.

Figure 2. Scheme of miRNA targeting of the tumor microenvironment through SMPD1 downregulation. Bioinformatic analyses were applied to elucidate the potential role of miR-15a, which putatively targets SMPD1, in the tumor endothelium.

Figure 3. Transfection of miRNAs induced at higher dose of radiation decreased cell viability to a magnitude similar to high dose radiation alone. CT-26 cells were transfected with respective miRNA reagents and compared to a control miRNA and/or non-transfected sample. 24h after transfection, cells were irradiated with either a 2 Gy or 10 Gy dose in a single fraction. 48h post radiation proliferation was measured using a luciferase based cell titer glo. Bars indicate means ± SEM of 3 technical replicate wells. One of at least two independent experiments is shown.

Figure 4. miRNA candidates targeting SMPD1 exhibit radiation dose-dependent differential expression at 6h post-IR in HUVECS. Fold change is indicated in colored cells relative to expression of the respective miRNAs in non-irradiated samples. Red = increased expression, Green = decreased expression.

Figure 5. miR-15a inhibition decreases EC proliferation and viability

Figure 6. A. Cell titer glo assay as described in Figure 5. Inhibition of miR-15a decreases cellular viability of HCT-116 colorectal cancer cells. B. Inhibition of miR-15a decreased cellular viability of CT-26 colorectal cancer cells Bars indicate means ± SEM of 3 technical replicate wells. One of at least two independent experiments is shown. C. CT26 tumors were implanted subcutaneously in Balb/C mice (n=14 mice per group). Two tumors per mouse were allowed to reach 300 mm3 volume, mice were randomly assigned to either a negative control inhibitor group or a miR-15a inhibitor (20mg/kg, i.s. in PBS). Mice were treated every 2 days for a total of three treatments. **p<0.01, ANOVA on the day 7 tumor volumes.

Conclusions and Future Directions

- CRC and vasculature exhibits differential radiation dose-dependent miRNA expression
- miRNAs can be modulated to mimic a high radiation dose environment
- Ongoing efforts include mechanistic elucidation of additional downstream target effectors of miR-15a inhibition and targeted delivery of miR-15a through endothelial selective 7C1 nanoparticles and nanophosphors

Acknowledgments and Research Team

S.A is supported by US NIH grant R01HL119262, a grant from the Medical Research Foundation and an innovative research grant from the American Heart Association.

References