Methionine intake modulates radiation damage in the gut

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Abstract

The use of radiation therapy in cancer is limited by normal tissue toxicity. Our goal is to increase the differential between the damage inflicted to the tumor relative to the normal surrounding tissue. Our previous work showed that high levels of dietary methionine exacerbate radiation damage in the gut. Methionine is an essential amino acid that also has a role as a methyl donor and in the synthesis of the antioxidant glutathione. Lower levels of methionine intake are associated with an increase in longevity in animal models. We also showed that reducing dietary intake of the essential amino acid methionine sensitizes tumors to radiation therapy without inducing weight loss. Furthermore, methionine restriction decreases markers of inflammation and increases markers of tight junction in the healthy murine gut. In this experiment, we irradiated the abdominal area with a single dose of 12.5 Gy X-ray using a Small Animal Radiation Research Platform (SARRP). Half of the mice remained on a standard diet (0.65% methionine) while the other half received a methionine-restricted diet (0.12% methionine) for one week preceding and one week following radiation. At one week after radiation, we observed evidence of gut protection from radiation in animals that were consuming a methionine-restricted diet versus a control diet. This indicates that lowering the amount of methionine in the diet of patients receiving cancer radiotherapy may help in mitigating side effects.

Background

- **Normal tissue toxicity limits the dose of radiation that can be used in patients with rectal cancer.**
- **Side effects from radiation can be severe and long-lasting.**
- **Lowering dietary methionine was previously shown to improve tight junctions in the gut.**

Mutations associated with radioresistance modulate methionine-related metabolites

HCT116 WT vs G13D

Superpathway	Subpathway	Biochemical	Fold Change
Amino Acid	Glutamate Metabolism	S-1-pyrroline-5-carboxylate	1.85
Amino Acid	Histidine Metabolism	1-methylhistidine	0.49
Amino Acid	**Methionine, Cysteine, SAM and Taurine Metabolism**	**S-methylmethionine (SAM)**	**0.64**
Amino Acid	**Methionine, Cysteine, SAM and Taurine Metabolism**	**S-adenosylhomocysteine (SAH)**	**0.65**
Amino Acid	**Methionine, Cysteine, SAM and Taurine Metabolism**	**cysteine**	**0.72**
Amino Acid	Urea cycle; Arginine and Proline Metabolism	N,N,N-trimethyl-alanylproline betaine (TMAP)	2.42
Lipid	Sphingolipid Synthesis	sphingadienine	1.56
Lipid	Sphingosines	sphingosine	1.40
Lipid	Sphingosines	hexadecasphingosine (d16:1)*	1.42
Lipid	Sphingosines	heptadecasphingosine (d17:1)	1.55
Nucleotide	Pyrimidine Metabolism, Uracil containing	uracil	0.56
Cofactors and Vitamins	Riboflavin Metabolism	flavin adenine dinucleotide (FAD)	1.54
Xenobiotics	Food Component/Plant	beta-guanidinopropanoate	1.96
Xenobiotics	Chemical	thioproline	0.70

Low methionine decreases DNA damage in epithelial cells

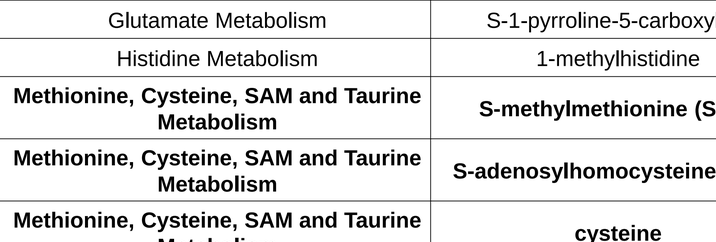

Figure 1. Hs738 human intestinal epithelial cells were cultured for 24h in media containing varying concentrations of methionine (control = 200 μM) then irradiated with 5 Gy X-ray and harvested after 6 hours. Lower concentrations of methionine lead to less γH2A.X, a marker of DNA damage, in the irradiated cells.

Methionine and KRAS interact in DNA damage

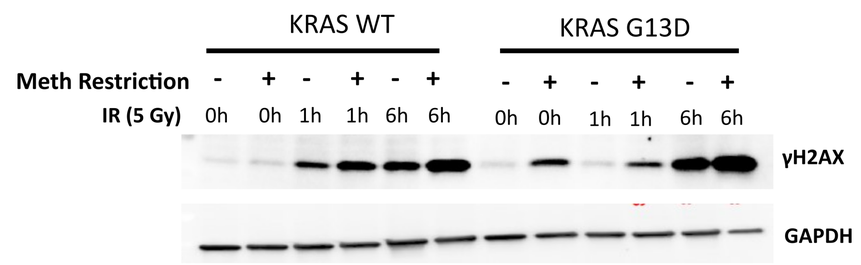

Figure 2. There was an increase in the marker of DNA damage γH2A.X in HCT116 cells grown in low methionine (+) and exposed to 5 Gy of radiation. This was exacerbated in KRAS mutant cells (G13D).

Limiting dietary methionine improves the response to radiation in KRAS mutant tumors

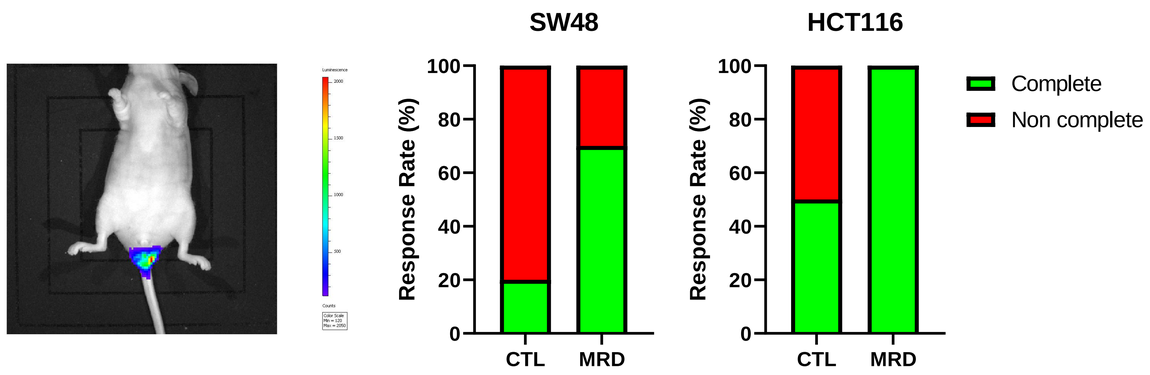

Figure 3. The KRAS G13D mutant cells were injected intrarectally. The mice were consuming a control diet or a diet reduced in methionine and received 5 consecutive daily fractions of 5 Gy of radiation. Tumor progression was evaluated by luminescence.

Methionine modulates healthy tissue damage

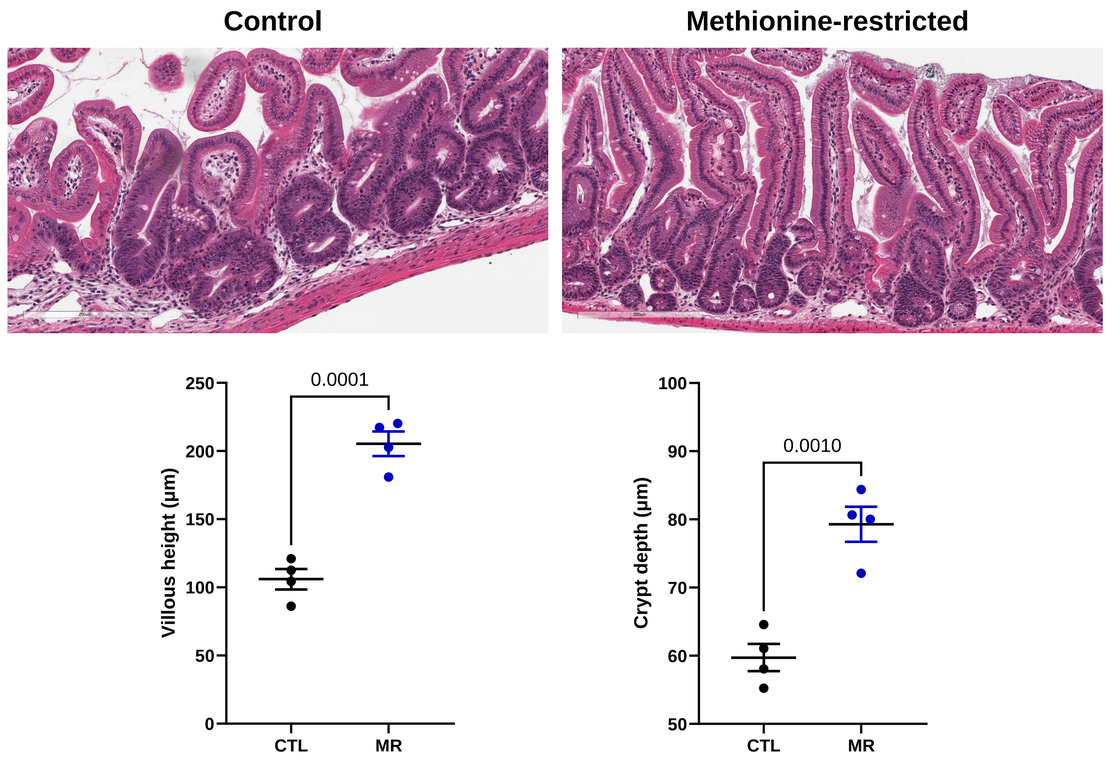

Figure 4. Limiting methionine, which sensitizes tumors, also protects the healthy intestinal lining. Mice received a single fraction of 12.5 Gy of radiation to the abdomen. The epithelium of animals that were methionine-restricted diet showed a limited amount of damage to the epithelium of the proximal jejunum (right), reflected by the increased villous height and crypt depth at day 7 post-radiation.

Conclusions

- **The radioresistant KRAS G13D mutation was associated with changes in methionine-related metabolites.**
- **Less marker of DNA damage was observed in non-cancer cells grown in low methionine compared to control media.**
- **A lower methionine availability restored radiosensitivity in KRAS mutant cells.**
- **More animals with a KRAS mutant tumor showed a complete response to radiation in the group that was methionine-restricted.**
- **Restricting methionine also protected the healthy gut mucosa.**

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