Molecular and Histopathological Changes in Mouse Intestinal Tissue after Proton Exposure

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Background

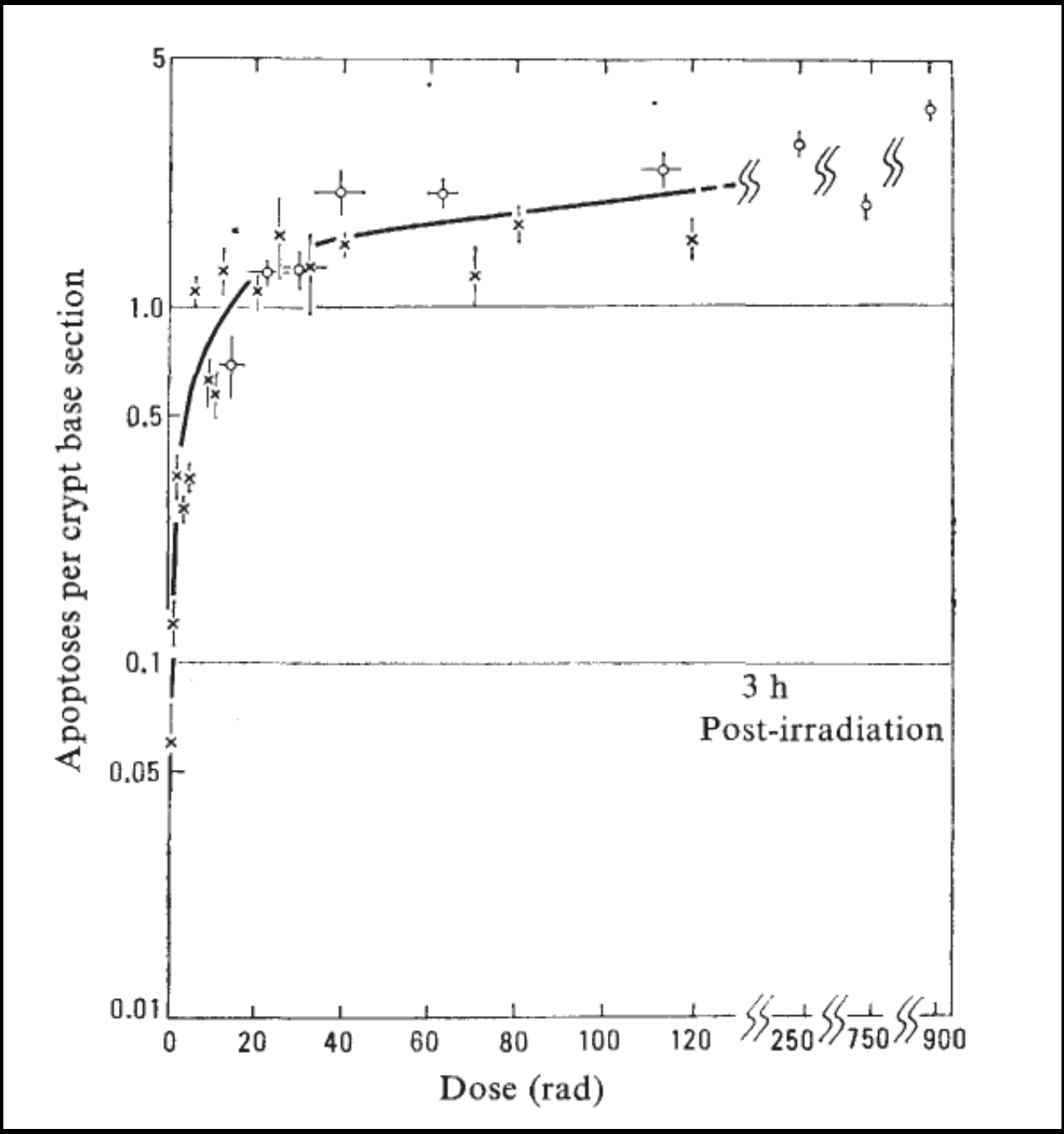
- Well established that protons are the most abundant particles in space
- Astronauts on long duration missions could face unpredictable proton exposures due to solar particle events
- Little work has been done to address the biological consequences of whole body-proton irradiation

Background

- We know that gamma irradiation causes a marked increase in apoptotic lesions in the small intestine

- These lesions peak between 3-6 hours post-irradiation and follow a dose-dependent relationship

- The number of lesions present do not seem to follow a strictly linear relationship


Apoptoses per crypt base section
5
1.0
0.5
0.1
0.05
0.01
3 h
Post-irradiation
0
20
40
60
80
100
120
250
750
900
Dose (rad)

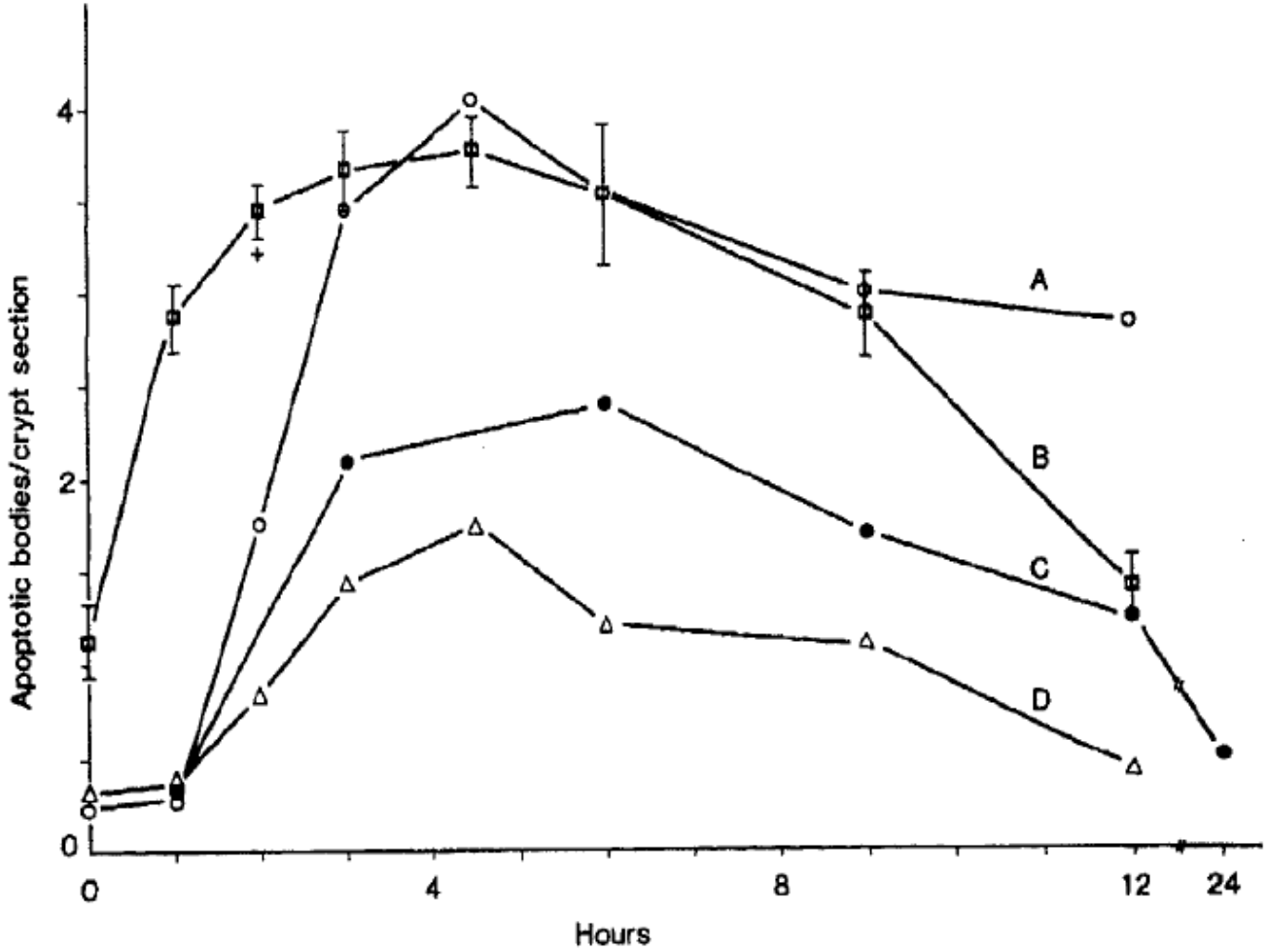


Figure 1. Time course of the appearance and disappearance of apoptotic bodies in crypt sections. All irradiations in Manchester. Curve A and (○), after 36 cGy ^{137}Cs γ-rays at 450 cGy per min. Curve B and (□), after 36 cGy ^{60}Co γ-rays at 0·27 cGy per min. Representative standard errors are shown on curve B. Curve C and (●), after 22 cGy ^{137}Cs γ-rays at 450 cGy per min. Curve D and (△), after 5 cGy ^{60}Co γ-rays at 0·27 cGy per min. Note break in time scale between 12 and 24 hours. All times measured from the end of irradiation.

Methods

- This project entailed the use of a BALB/C mouse model undergoing whole body exposure with 250 MeV of proton radiation

- 4 groups of three mice each
  - 0 Gy (sham)
  - 0.1 Gy
  - 1 Gy
  - 2 Gy

- The small intestine was chosen as our organ of interest due to its radio-sensitivity

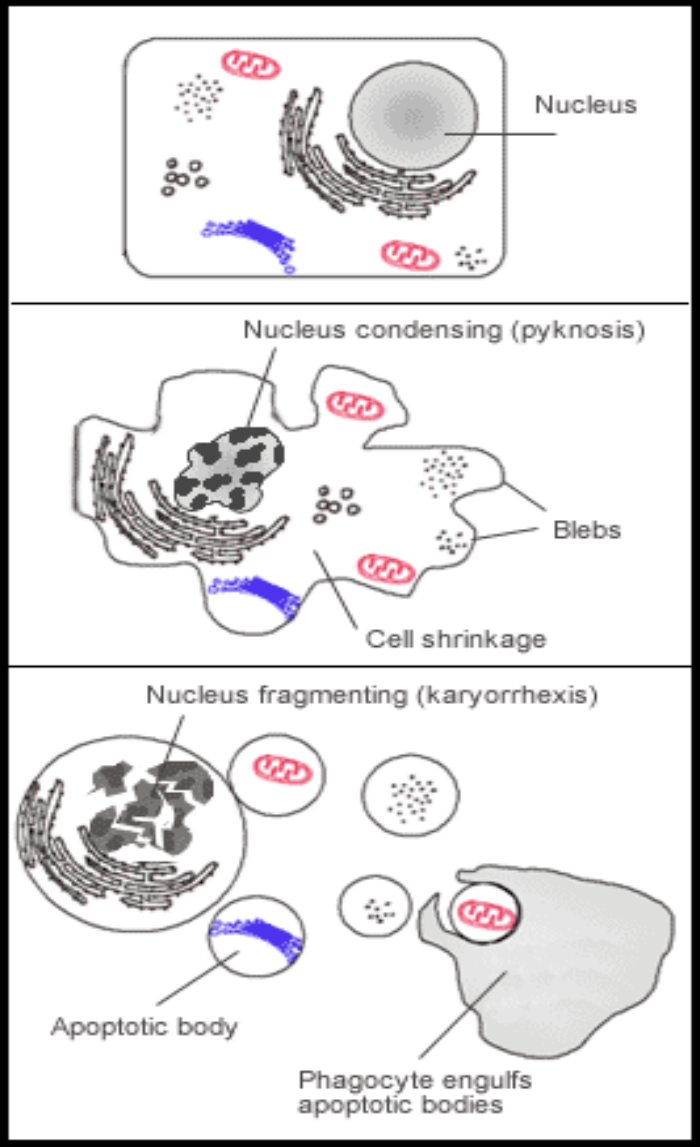
Methods

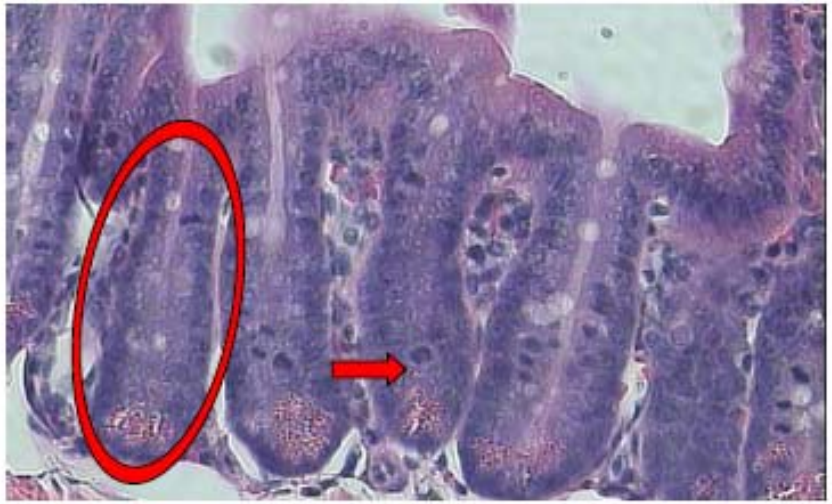
- Animals were sacrificed four hours post-irradiation and the GI tract was isolated
- Tissue was fixed in formalin for histopathological analysis
  - samples were embedded in paraffin and sectioned for slides
  - standard H&E staining was used to observe any morphologic changes present
- Or snap-frozen in liquid N_2 for RNA isolation
  - real-time PCR was used to look at gene expression changes in 84 genes among various apoptotic pathways

Histopathology

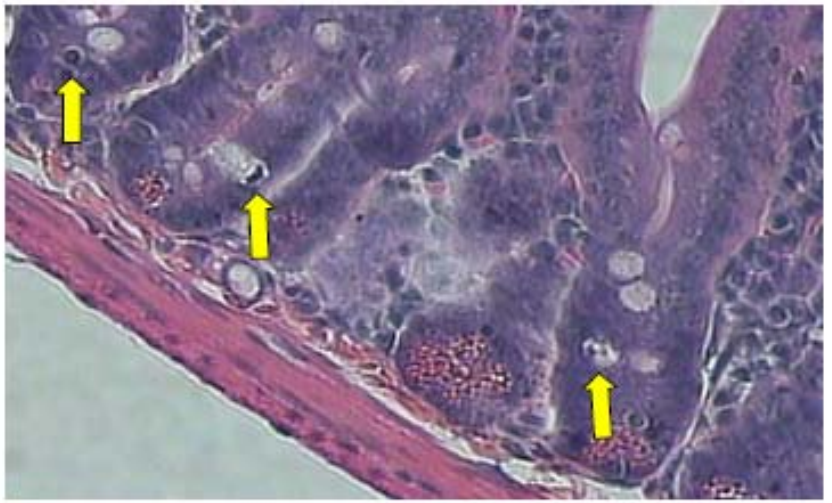
- Apoptotic lesions in the duodenum of the small intestine were visually quantified on 20 crypts selected at random for each animal

- A lesion was identified using standard criteria: a cell undergoing pyknosis or karyorrhexis

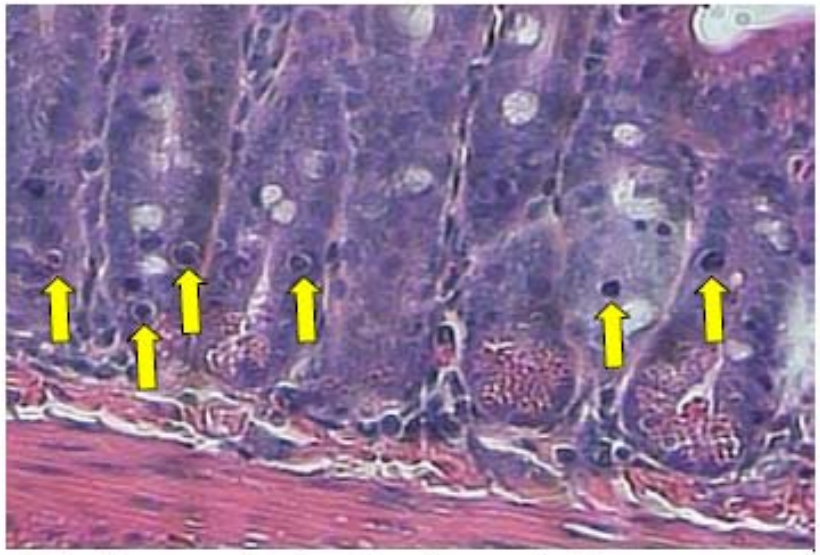




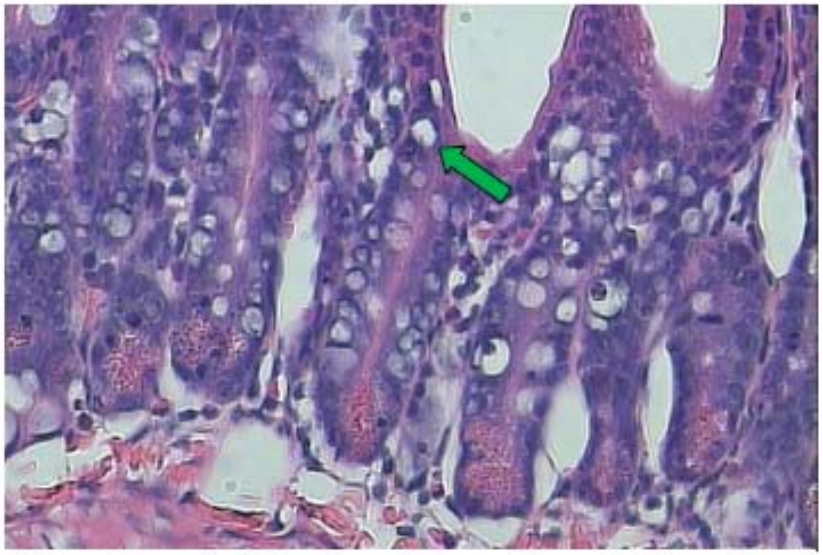
CONTROL. The red arrow is pointing to a typical mitotic cell in a crypt of tissue of the duodenum. The cell appears darker as the chromatin has condensed in preparation for cell division. The red circle identifies one crypt of the tissue.



0.1 Gy. The yellow arrows are pointing to typical apoptotic lesions. Note the condensed and fragmented nuclei surrounded by swelling of the cell. The damaged cells are in close proximity to the basal area of the crypts.



1 Gy. It appears that there is an increase in damage as displayed by the yellow arrows. There is also a noticeable decrease in the number of mitotic cells.



2 Gy. Apoptotic lesions have increased dramatically and are now present in all areas of the crypts, approaching the villi. A preapoptotic lesion displaying extreme swelling of the cell is shown with the green arrow.

Quantification of Lesions

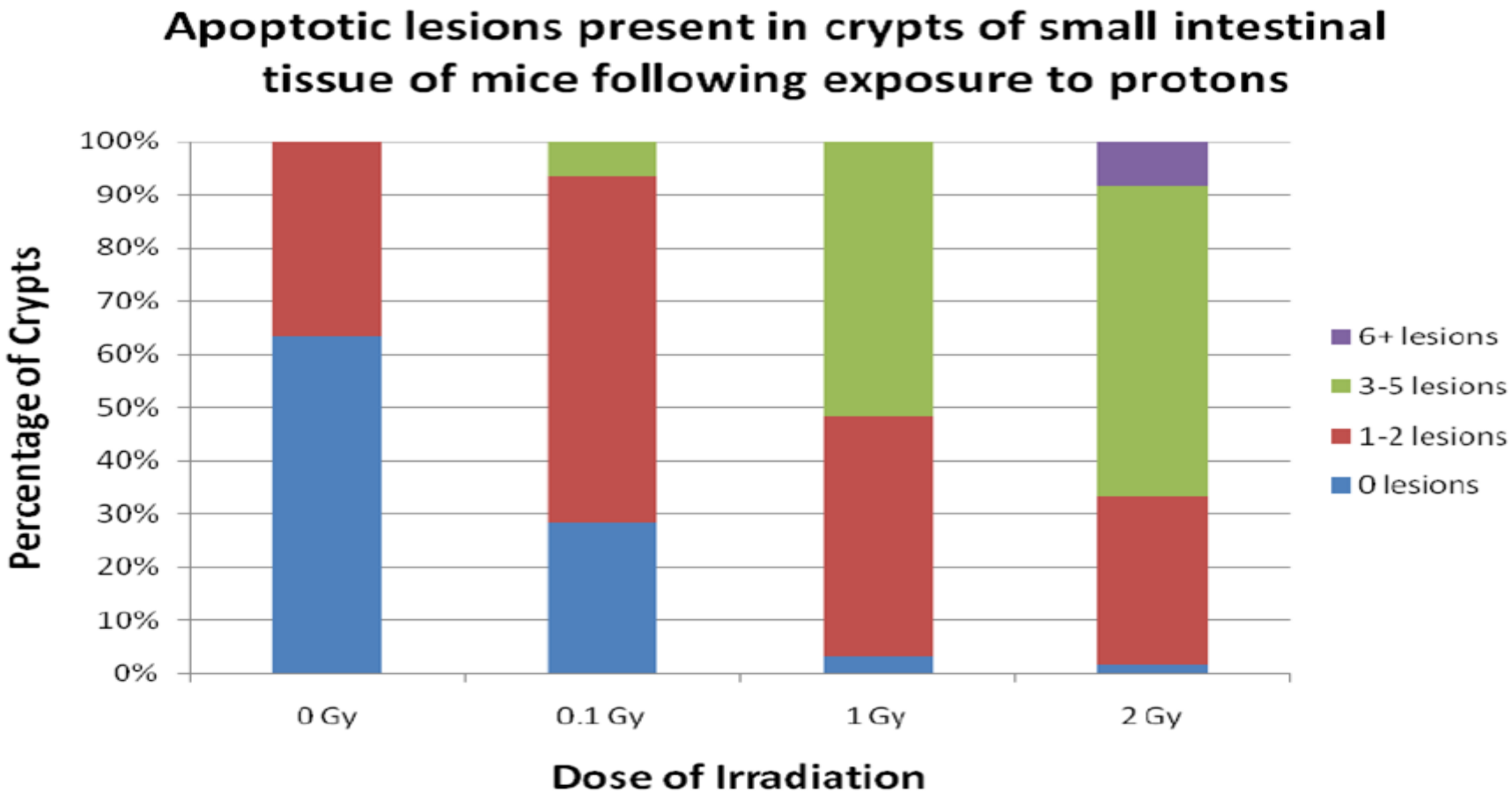


Figure 1. The percentages of crypts containing varying quantities of apoptotic lesions are shown for each dose of proton exposure.

Quantification of Lesions

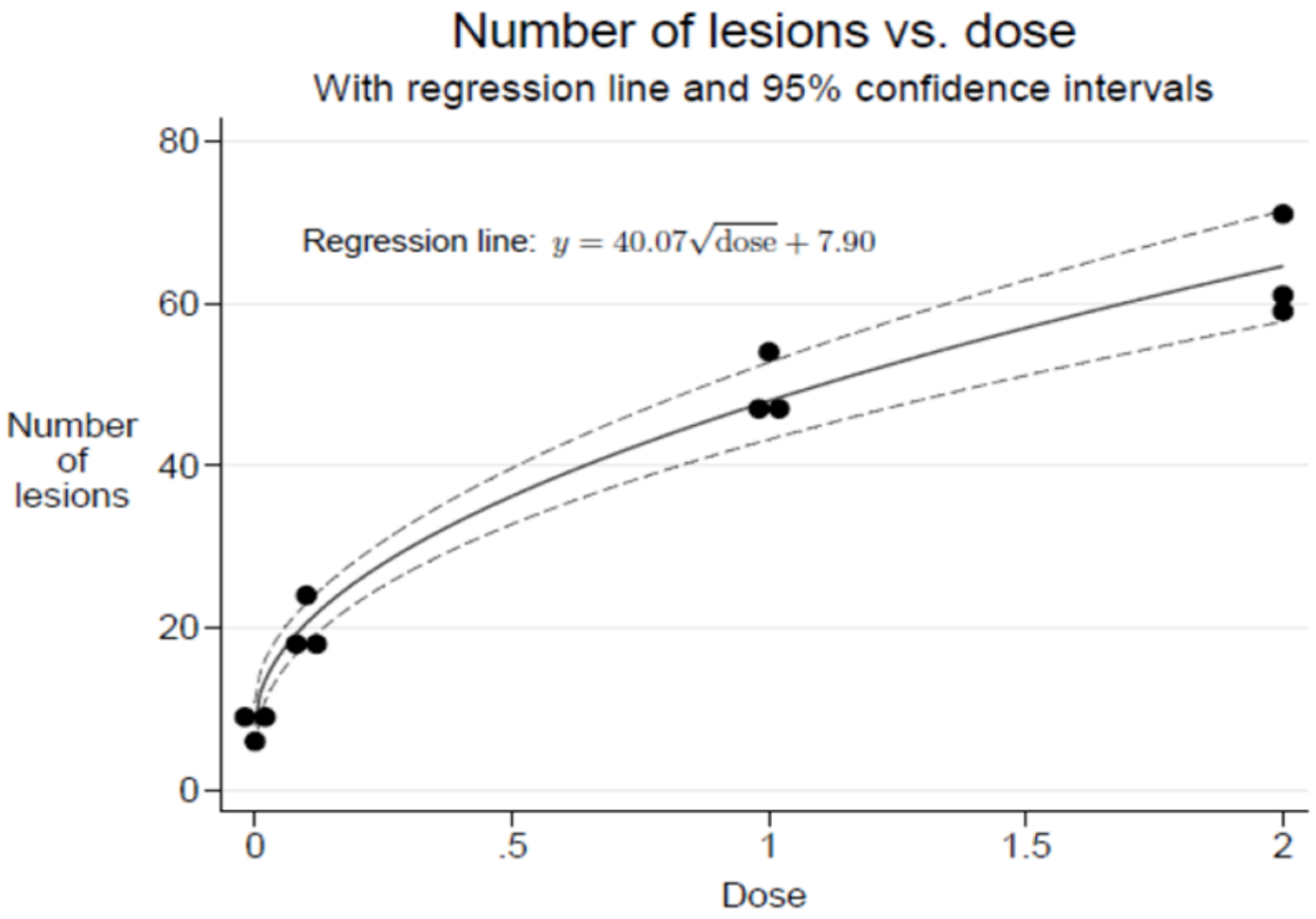


Figure 2. Lesions appear to increase with increasing dose of proton exposure (slope: $p < 0.001$, $\alpha=0.05$; 95% CI: 34.47, 45.67 and intercept: $p < 0.001$, $\alpha=0.05$; 95% CI: 5.00, 10.79). Some values of dose have been modified slightly to prevent overlap.

Gene expression alterations

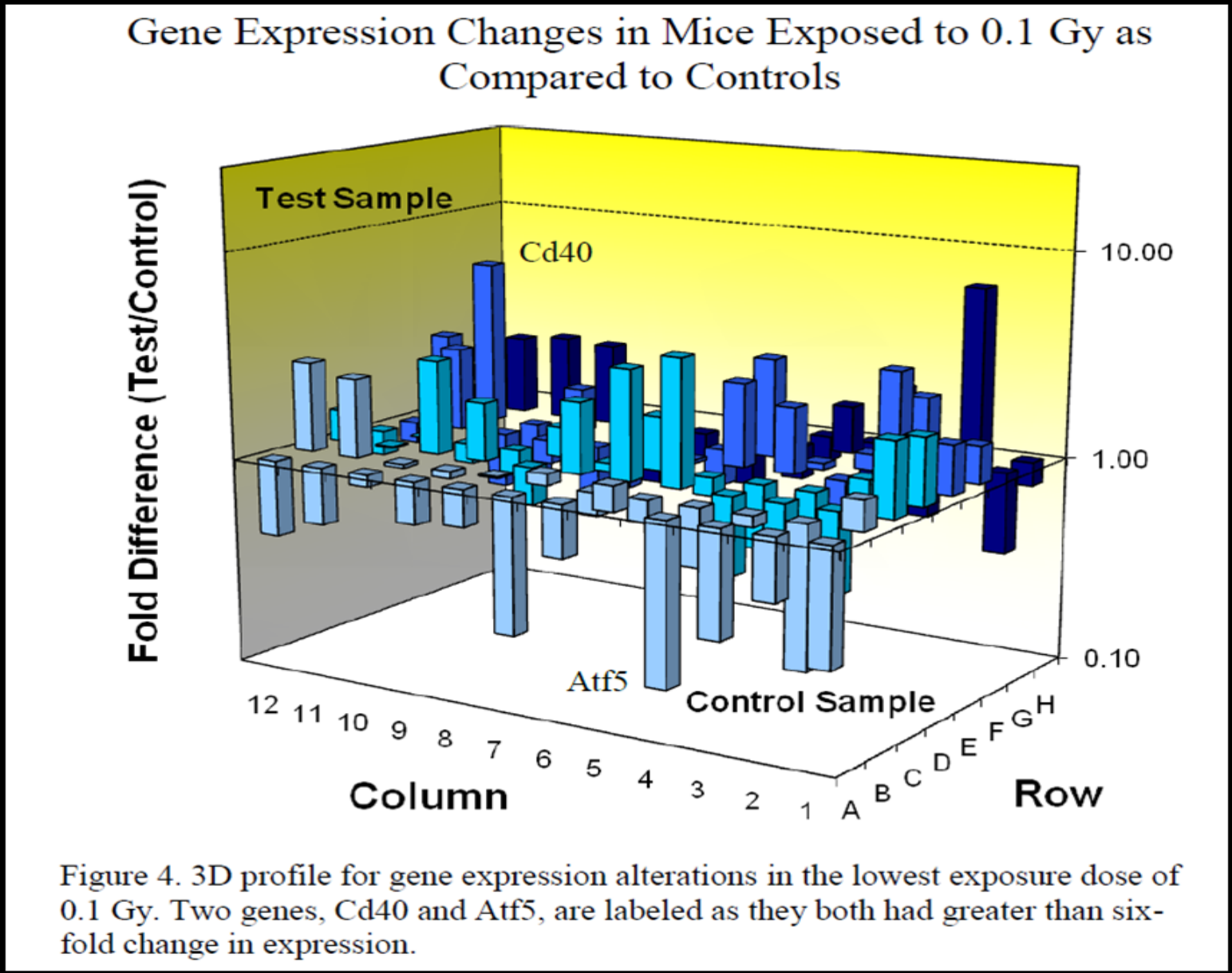


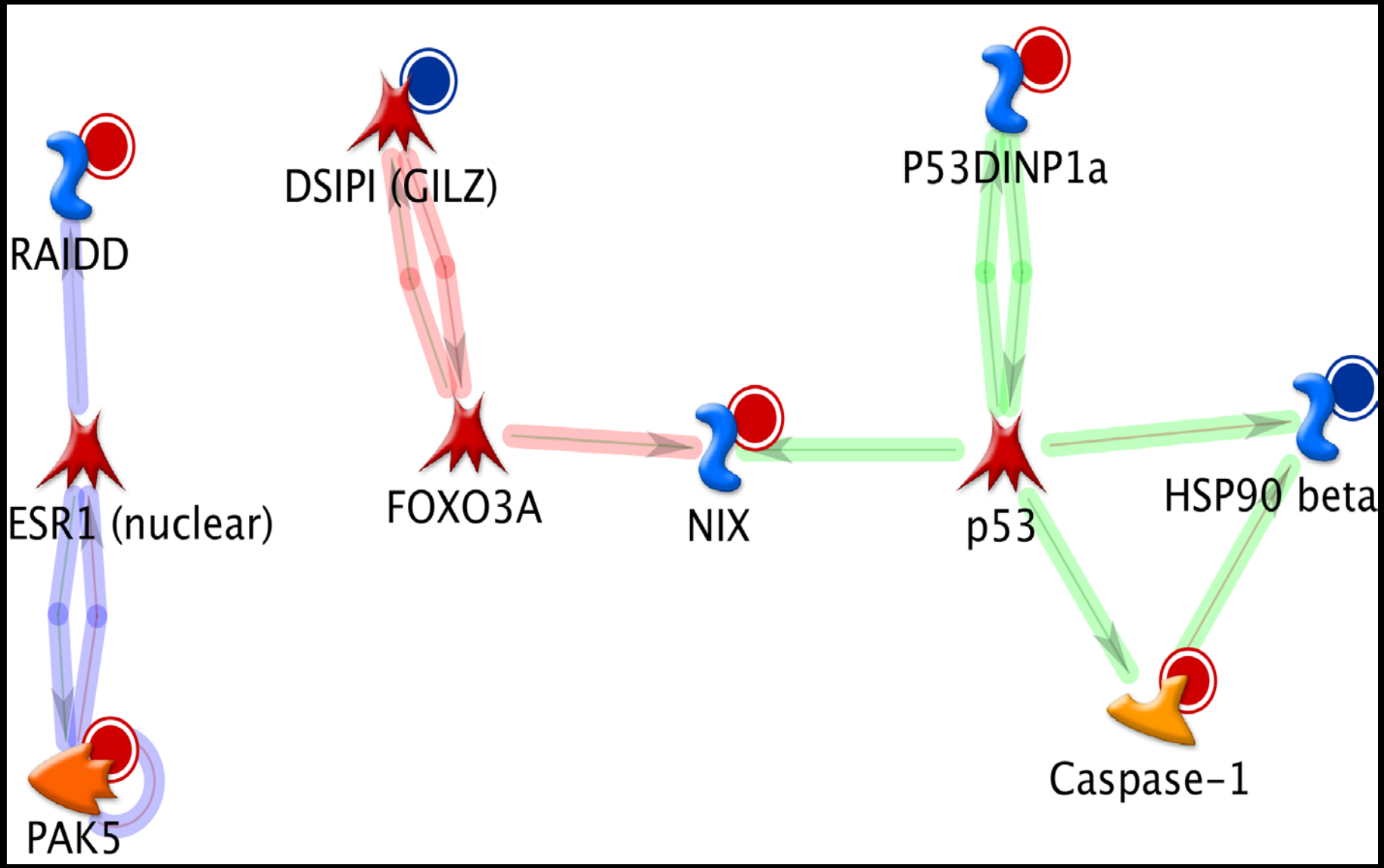
Figure 4. 3D profile for gene expression alterations in the lowest exposure dose of 0.1 Gy. Two genes, Cd40 and Atf5, are labeled as they both had greater than six-fold change in expression.

Gene expression alterations

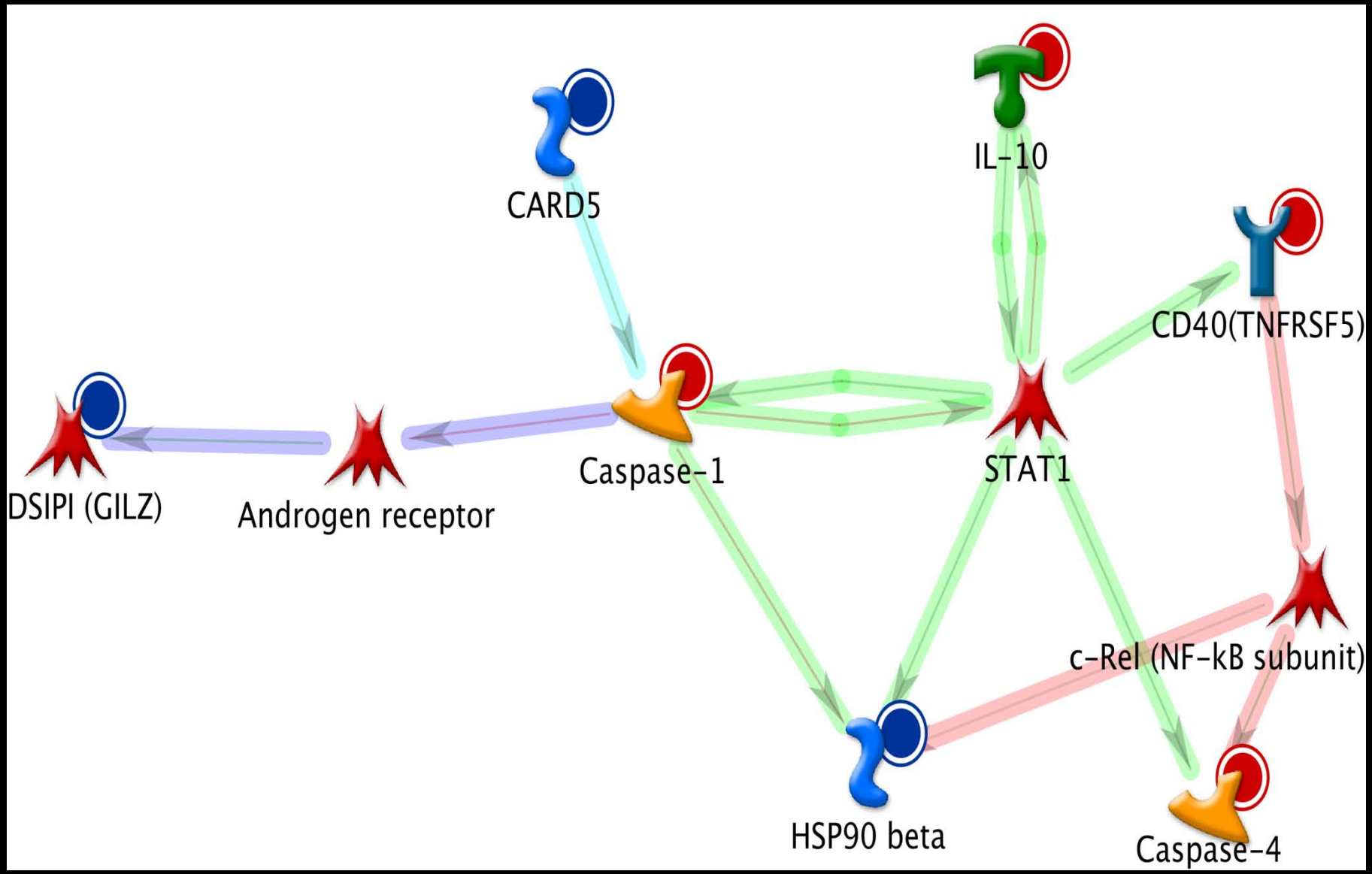
Symbol	0.1 Gy fold change	1 Gy fold change	2 Gy fold change
Atf5	-6.0	-4.3	-1.4
Bnip3l	2.7	1.7	1.3
Bok	2.3	1.1	1.4
Casp1	4.1	1.4	1.6
Casp4	2.9	2.1	2.4
Cidea	-2.0	-4.0	-1.6
Tsc22d3	-1.0	-4.2	-4.3
Il10	2.1	2.3	1.7
Pak7	2.5	1.2	1.5
Pycard	1.2	1.0	-2.3
Rnf7	3.0	1.5	1.2
Cd40	6.2	3.7	4.3
Tnfsf12	-2.5	-2.4	-1.1
Trp53inp1	2.5	3.3	4.1
Hprt1	6.5	3.2	2.6
Hsp90ab1	-2.2	-3.7	-2.7

Table 1.Mouse apoptosis gene expression fold changes by dose and gene. Significant changes are highlighted in red ($p < 0.05$, $\alpha=0.05$). A positive fold change indicates increased expression as compared to control specimens (0 Gy) while a negative value is indicative of down-regulation as compared to controls.

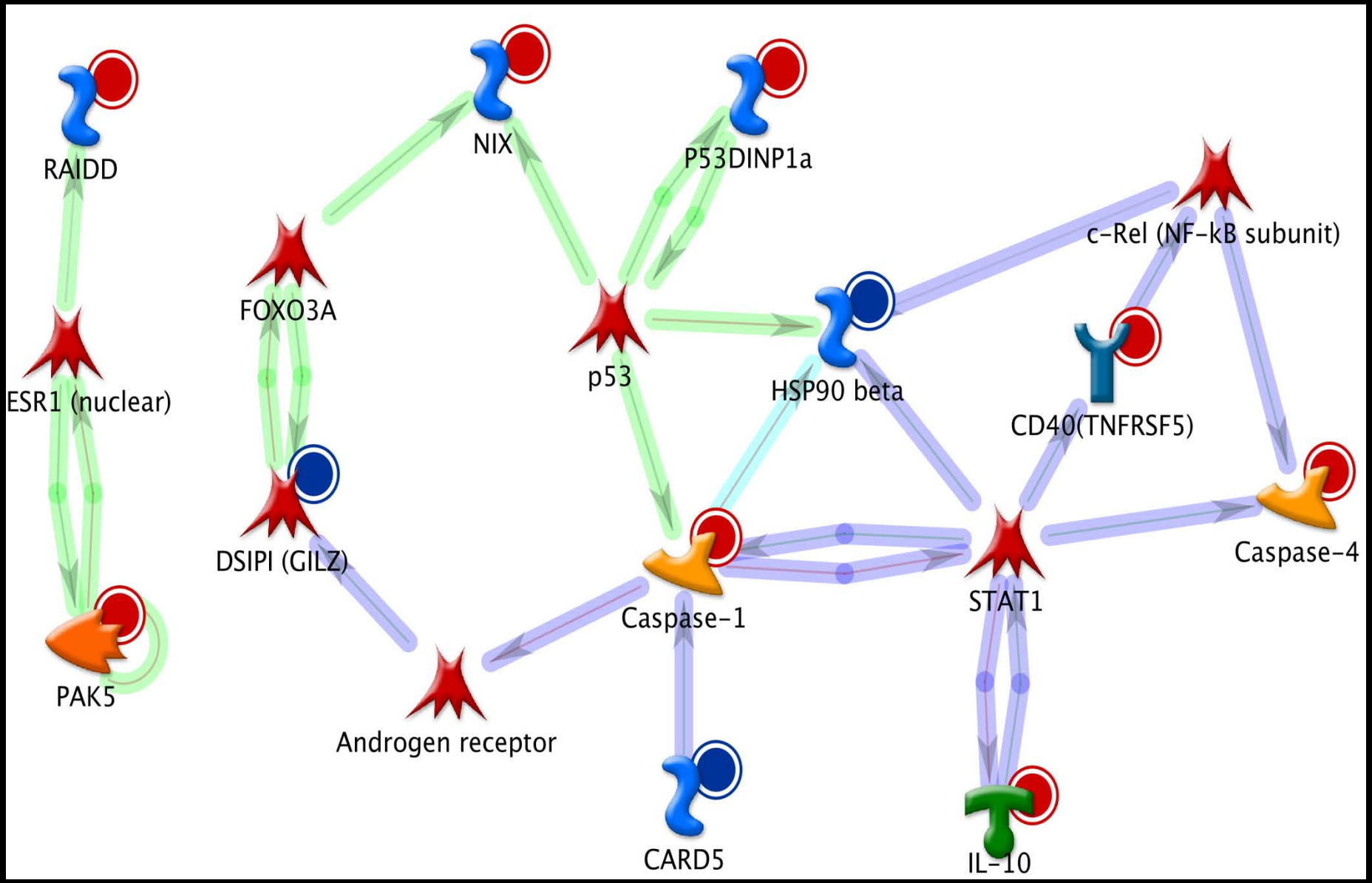
Pathway Analysis



Pathway Analysis



Pathway Analysis



Conclusions

- Whole body exposure to protons in mice causes significant apoptosis in the crypts of the small intestine
- Increasing numbers of crypts contained more apoptotic lesions as the dose of exposure increased
- 16 genes associated with apoptotic pathways were shown to have significantly altered expression as compared to control samples for at least one of the doses of proton exposure
  - 1 gene, Trp53inp1, was significantly up-regulated across all three doses.

Conclusions

- Those animals exposed to 0.1 Gy of proton irradiation showed greater amounts of significant alterations in gene expression as compared to 1 Gy and 2 Gy exposures

- The differences in gene expression changes of low and high dose proton irradiated mice may offer insight into the molecular mechanisms of the possible high sensitivity at low proton doses

Conclusions

- RAIDD (CRADD) may be responsible for the hypersensitivity observed in the duodenum of mice exposed to low doses of protons
- Caspase-1 may also play a role in the hypersensitivity seen following proton irradiation at a dose of 0.1 Gy
- FOXO3A may be involved in the down-regulation of GILZ observed at high doses of proton exposure

Future Study

- This work could have important health implications for astronauts on potential missions that would go beyond low Earth orbit
- The hypersensitivity seen here at low dose exposure to protons could play a role in future risk-assessment of long duration space travel
- Currently, IHC is being performed to confirm some of the up- and down- regulations found using real-time PCR

Future Study

- siRNA could be used to determine the importance of some of the genes hypothesized to play differing roles following low and high doses of proton exposures
    - For example, ESR1 as it affects RAIDD
- We would like to continue this work with intentions of comparing damage and repair markers due to proton and gamma irradiation in a time-dependent manner

Questions?

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