Effects of depleting NONO, a novel DNA repair factor, in human melanoma cells

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INTRODUCTION

Skin cancers can be caused by environmental factors such as prolonged sunlight exposure and ionizing radiation, including space radiation. Melanoma is a lethal form of skin cancer arising most commonly from exposure to sunlight. Although curable when detected early, melanoma often escapes detection until it has already begun to spread. Advanced melanomas are resistant to most forms of treatment. For this reason, better treatments are needed.

It has recently been reported that melanomas overexpress a protein called NONO (p54nn). NONO has a number of functions in the cell, depending on what other proteins are bound to it. One of these functions is in promoting repair of DNA double-strand breaks through a specific non-homologous end-joining pathway (NHEJ), shown in Figure 1.2 It is not known, however, whether the overexpressed NONO in melanomas is related to enhanced DNA repair or to one of the other functions of the protein. Our goal was to find out if reducing the level of NONO in melanoma cells would delay DNA double-strand break repair.

To do this, I used a marker of unrepaired DNA double-strand breaks called γ-H2AX. This modified histone can be detected using immunofluorescence staining and will appear as dots (or foci). With this method, we can indirectly measure DNA damage that can be quantified. We depleted (or “knocked down”) the amount of NONO using RNA interference technology then assessed the difference in repair kinetics between control and knockdown groups.

RESULTS

Cell lines: 1. A375; 2. MeWo; 3. SK-MEL2; 4. SK-MEL28

Table 1. Statistical analysis of data from Figure 3 and SK-MEL2 histograms (not shown).

<table>
<thead>
<tr>
<th>Recovery Period after 1 Gy</th>
<th>Average Foci for Control</th>
<th>Average Foci for Knockdown</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not irradiated</td>
<td>7.7 ± 6.6</td>
<td>7.1 ± 7.6</td>
<td>0.67</td>
</tr>
<tr>
<td>30 minutes</td>
<td>9.1 ± 7.7</td>
<td>9.7 ± 9.7</td>
<td>0.06</td>
</tr>
<tr>
<td>2 hours</td>
<td>9.0 ± 7.7</td>
<td>19.4 ± 9.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>4 hours</td>
<td>7.5 ± 5.5</td>
<td>16.3 ± 11.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Knockdown</td>
<td>5.1 ± 5.1</td>
<td>5.8 ± 4.9</td>
<td>0.51</td>
</tr>
<tr>
<td>30 minutes</td>
<td>19.4 ± 8.9</td>
<td>20.8 ± 9.2</td>
<td>0.36</td>
</tr>
<tr>
<td>2 hours</td>
<td>8.8 ± 6.9</td>
<td>15.1 ± 7.7</td>
<td>&lt;0.0002</td>
</tr>
<tr>
<td>4 hours</td>
<td>3.9 ± 3.5</td>
<td>10.5 ± 7.6</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Figure 2. Verification of shRNA-mediated NONO knockdown by Western blot. Four cell lines were tested (see key). The graph on the right shows quantification of the amount of NONO expression in control (blue) and knockdown (red) groups. The A375 and SK-MEL2 LINES were selected for further study based on efficient knockdown.

Figure 3. Representative immunofluorescence images. A375 cells were fixed after 4 hours of recovery and stained with primary anti-NONO and anti-γ-H2AX antibodies along with appropriate dye-conjugated secondary antibodies (green is anti-NONO and red is anti-γ-H2AX). Cells were counter stained for DNA with DAPI (blue). Each row shows the same field in blue, green, and red channels, respectively. Note the persistence of γ-H2AX staining in knockdown group, particularly in cells with the most efficient knockdown. Scale bar is 15 µm.

Figure 4. Histograms showing distribution of ‘γ-H2AX foci per cell in A375 cells at various times of recovery post-irradiation. Approximately 50 cells in each treatment group were randomly selected and the number of foci per cell was manually counted. The graphs show similar numbers of background foci in non-irradiated control (blue) and knockdown (red) groups. Irradiation induced a similar number of foci as measured 30 minutes after irradiation. However, as repair progressed at 2 and 4 hours, a difference between control and knockdown groups was evident, with repair delayed in the knockdown group. Similar results were obtained for the SK-MEL2 line (not shown).

DISCUSSION AND CONCLUSIONS

Our findings suggest that there is a difference in melanoma cell response to radiation depending on the amount of NONO present in the cell. The Western blot in Figure 2 shows that virus mediated knockdown reduced NONO expression by up to 80%. Two cell lines with the most effective knockdown were selected for further studies of DNA repair kinetics using γ-H2AX as a marker. Previous studies have shown a 1:1 correspondence between these foci and unrepaired double-strand breaks.

After IF staining and manual foci counting, we were able to visualize a clear difference between control and knockdown populations. With DAPI, which stains total DNA, all cell nuclei light up equally. With anti-NONO, the knockdown cells are mostly dimmer than the control cells. Conversely, with anti-γ-H2AX, the knockdown cells are much brighter after 4 h recovery.

I counted foci in approximately 50 cells per experimental group. Results with one of the cell lines is shown as histograms in Figure 4. A statistical analysis of results with both cell lines is shown in Table 1. According to these results, control and knockdown groups have a similar number of background foci in the absence of radiation. The initial number of foci induced (measured at 30 min post-irradiation) was also similar. However, the foci disappeared much more slowly in the knockdown group. Table 1 indicates a substantial difference between control and knockdown groups at 2 and 4 hours of recovery. P-values were significant based on a t-tailed Student’s t-test with α = 0.05. This further supports our hypothesis that the high levels of NONO in melanoma cells promote efficient DNA double-strand break repair.

This work shows a promising future for the clinical application of treating melanoma cancer in patients. If melanoma can be sensitized to therapy by knocking down NONO expression, or using a pharmacological inhibitor of NONO activity, then it could potentially help treat patients in a more efficient way than the methods we have at present.

 METHODS

- Knockdown NONO expression using short hairpin RNA-expressing lentivirus
- Check knockdown efficiency by Western blot
- Irradiate cells with 1 Gy of 127Cs γ-rays (produces about 30 double-strand breaks per cell)
- Fix cells after 30 minutes, 2 hours, 4 hours, and 8 hours of recovery to measure repair kinetics
- Perform immunofluorescence (IF) staining to observe γ-H2AX foci

REFERENCES