Possibilities for Treatment of Melanoma: Effects on Cell Proliferation by Inhibition of Melanoma Chondroitin Sulfate Proteoglycan (MCSP) and B-Raf In Vitro.

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Introduction

- The MAP Kinase cell signaling pathway controls cell growth by allowing cells to divide only in the presence of growth factor.
- Growth factor binding to receptor turns on cell signaling cascade.
- This involves cytoplasmic proteins Ras, B-Raf, Mek, and Erk.
- In 60% Melanomas, B-Raf is found in a mutant form.
- Mutant B-Raf allows cell division to proceed in the absence of growth factor by constitutively activating Mek and Erk.
- MCSP is heavily expressed in melanoma while rarely expressed in normal melanocytes.
- MCSP expression is correlated with constitutively active Erk 1,2.
- Thus, it is of clinical significance to consider MCSP and B-Raf as cellular candidates for treatment of melanoma.

Hypothesis

- VM1341D is MCSP/B-Raf+ so MCSP antibody and Bay 43-9006 should inhibit cell proliferation. Dual treatment should have additive inhibitory effect.
- A375 is MCSP/B-Raf- so we expect that MCSP antibody should reduce the number of viable cells. However, Bay 43-9006 should not inhibit cell division. Dual treatment should have an additive effect.

Materials

- Melanoma Cell Line VM1341D: Expresses MCSP and mutant B-Raf (MCSP/B-Raf+).
- Melanoma Cell Line A375: Expresses MCSP and normal B-Raf (MCSP/B-Raf-).
- Bay 43-9006: Bayer: 200 mg oral drug in the market for therapy against melanoma.
- MCSP An9body: Obtained from Soldano Ferrone M.D. Ph.D., University of Pittsburgh, Department of Immunology.

Methods

- To determine the effects on melanoma cell proliferation by simultaneous inhibition of MCSP and B-Raf using MCSP antibody and RAF kinase inhibitor Bay 43-9006 vs singular inhibition of MCSP or B-Raf.
- Thus, we are interested in comparing the therapeutic efficacy of Bay 43-9006 and MCSP antibody by co-treatment vs singular treatment.

Results on Cell Line WM1341D (MCSP/B-Raf+)

- Treatment with MCSP Antibody

<table>
<thead>
<tr>
<th>Concentration (uM)</th>
<th>0</th>
<th>0.5</th>
<th>1</th>
<th>2.5</th>
<th>5</th>
<th>10</th>
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</thead>
<tbody>
<tr>
<td>Cell Viability (%)</td>
<td>110</td>
<td>100</td>
<td>90</td>
<td>80</td>
<td>70</td>
<td>60</td>
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- Co-Treatment with SuM Antibody and Bay 43-9006 vs Bay 43-9006

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Results on Cell Line A375 (MCSP/B-Raf-)

- Treatment with MCSP Antibody

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- Co-Treatment with SuM Antibody and Bay 43-9006 vs Bay 43-9006

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Conclusion

- Administration of MCSP antibody can be used to sensitize wild type B-Raf cell line expressing MCSP to Bay 43-9006 for greater inhibition of cell proliferation (figure 5c).
- However, MCSP antibody addition to cell line co-expressing MCSP/BRAF with Bay 43-9006 treatment has less of an inhibitory effect than single administration (figure 4c).
- Bay 43-9006 is not selective for B-Raf as shown by its inhibitory effects on wild type B-Raf.

Implications

- Bay 43-9006 is used in the treatment of various cancers.
- A new generation of B-Raf specific inhibitors, PLX4032 and AZ628, are under evaluation in clinical trials specific for melanoma.
- However, after continuous exposure, melanomas have been shown to gain resistance to these drugs through additional B-Raf mutations.
- Thus there is greater need for research to evaluate MCSP’s modualtion of Erk1/2 for re-sensitizing mutant cells resistant to B-Raf inhibitors.

Literature