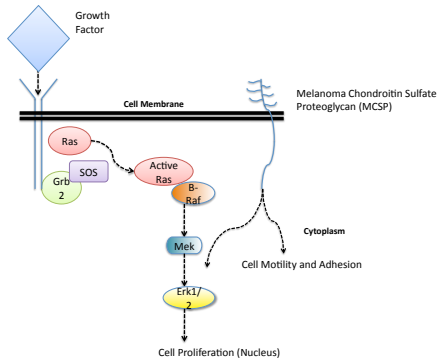
Possibilites for Treatment of Melanoma: Effects on Cell Proliferation by Inhibition of Melanoma Chondroitin Sulfate Proteoglycan(MCSP) and B-Raf In Vitro.

Omar G. Cespedes, Jianbo Yang, Kaoru Terai, Soldano Ferrone, Xinhui Wang, James B. McCarthy, Arkadiusz Z. Dudek
Division of Hematology, Oncology and Transplantation (HOT), University of Minnesota, Minneapolis, MN

Introduction

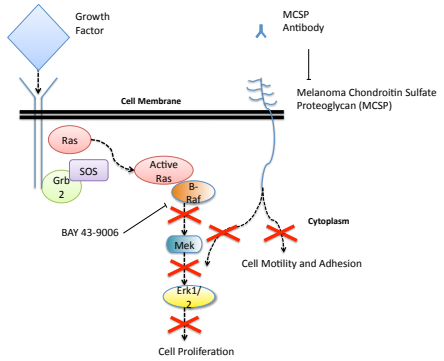
- The MAP Kinase cell signaling pathway controls cell-growth by allowing cells to divide only in the presence of growth factor.
- Growth factor binding to receptor turns on cell signaling cascade
- This involves cytplasmic proteins Ras, B-Raf, Mek and Erk.
- In 60% Melanomas B-Raf is found in a mutant form.
- Mutant B-RAF allows cell division to proceed in the absence of growth factor by constitutively activating Mek and Erk1,2.
- 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.



1. Cell proliferation proceeds through the Map- Kinase pathway. Growth factor binding in the cell membrane liberates the cytoplasmic protein Ras from scaffolding proteins Grb 2 and SOS and activates it into Active Ras. Subsequently, Active Ras turns on B-Raf which then activates Mek and Erk1/2. Erk 1/2 can then enter the nucleus to turn on specific genes involved in cell proliferation.In 60% of Melanomas, B-Raf is in a mutated form which allows proliferation to continue in the absence of growth factors. Normal melanocytes express little MCSP while heavy expression is seen in melanoma. The cell surface proteoglycan modifies cell adhesion and influences cell growth as it sustains constitutive activation of Erk1/2 downstream of B-Raf.

Our Goals

- 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 Bay43-9006 and MCSP antibody by co-treatment vs singular treatment.



2. Treatment with Bay 43-9006 selectively inhibits B-Raf. MCSP antibody binds to MCSP preventing it from exerting its control on cell proliferation through Erk1/2 activation. Thus, we expect that treatment with Bay 43-9006 on cells with mutant B-Raf should inhibit cell proliferation while having minimal effect on cells with wild-type B-Raf. Likewise, treating cells expressing MCSP with MCSP antibody should reduce the number of viable cells.

Hypothesis

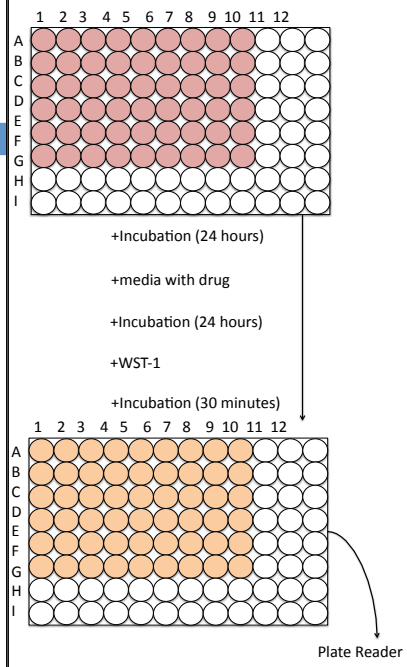
- WM1341D is MCSP$^+$/B-Raf$^-$ so MCSP antibody and Bay 43-9006 should inhibit cell proliferation. Dual treatment should have additive inhibitory effect.
- A375 is MCSP$^+$/B-Raf^{wt} so we expect that MCSP antibody should reduce the number of viable cells. However, Bay 43-9006 should not inhibit cell division. Dual treatment should have an additive effect.

Materials

- Melanoma Cell Line WM1341D.
  Expresses MCSP and mutant B-Raf (MCSP$^+$/B-Raf$^-$)
- Melanoma Cell Line A375.
  Expresses MCSP and normal B-Raf(MCSP$^+$/B-Raf^{wt})
- Bay 43-9006
  Bayer. 200 mg oral drug in the market for therapy against various cancers as a Raf-1 kinase inhibitor. Also shows inhibition of PDGF and VEGF receptors on blood vessels.
- Melanoma Chondroitin Sulfate Proteoglycan Antibody
  Obtained from Soldano Ferrone M.D. Ph.D
  University of Pittsburgh, Department of Immunology

Methods

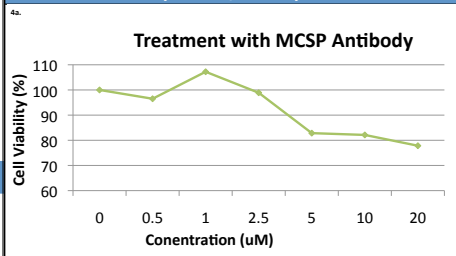
3a. Cells were plated on 96 well plate(pink wells A1 through G9) at a concentration of 3000 cells per well and incubated for 24 hours. Media was then removed and replaced with media with MCSP antibody and subsequently incubated for 24 hours. Cell viability was determined by adding WST-1 to wells and incubating for 30 minutes.



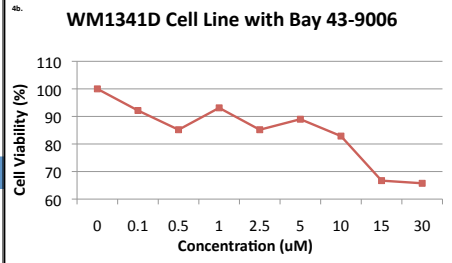
3b. WST-1 is taken in by viable cells and cleaved to formazan which gives off an orange hue. Absorbance is collected using a Plate Reader and quantized to determine viable cells across different concentrations of drug.

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

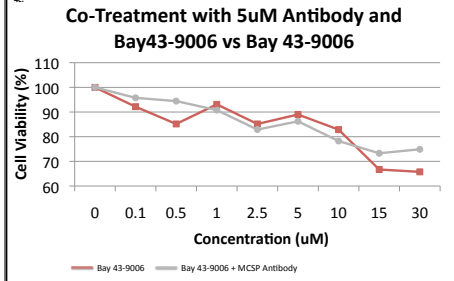
4a.



4b.

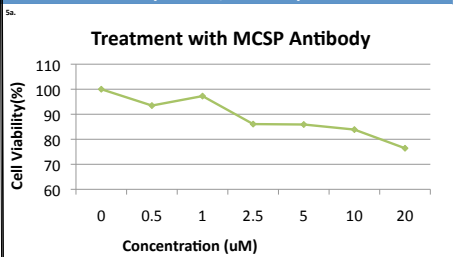


4c.

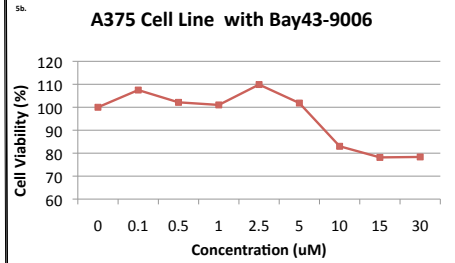


Results on Cell Line A375 (MCSP$^+$/B-Raf^{wt})

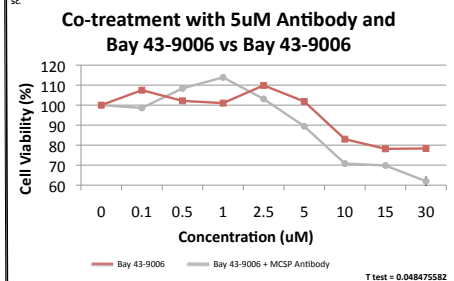
5a.



5b.



5c.



T test = 0.048475582

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 clinial 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 modulation of Erk1/2 for re-sensitizing mutant cells resistant to B-Raf inhibitors.

Literature

1. Jianbo Yang, et al." Melanoma Chondroitin Sulfate Proteoglycan enhances FAK and ERK activation by distinct mechanisms," *The Journal of Cell Biology* **165**, 881-891(2004).
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4. Samar Afify, et al. "Validation of liquid chromatography assay for the quantification of the Raf kinase inhibitor BAY 43-9006 in small volumes of mouse serum," *Journal of Chromatography B* **809**, 99-103(2004(