Title: A BACTERIAL-BASED PEPTIDE PROMOTING CELL ADHESION AND PREVENTING MIGRATION OF MELANOMA CELLS.

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Purpose:
The Shigella type III effector IpaA triggers the activation of the cytoskeletal linker vinculin to promote bacterial adhesion to cells1. Vinculin activation occurs through the concerted action of three vinculin binding sites (VBSs) present on IpaA1. Previous studies showed that cell ectopic expression of IpaA VBS1-2 inhibited melanoma cell migration and tumor formation in mice2. Here, we explored the role of a peptide containing all three IpaA VBSs in promoting cell adhesion and preventing cell migration and division.

Experimental Design:
Constructs containing the green fluorescent protein fused to IpaA VBSs (AVBSs) were transfected in cultured cells. Adhesion structures in transfected cells were analyzed for their size and stability by immuno-fluorescence following plating on various substrate stiffness, as well as by total internal reflection microscopy on live samples. The effects of AVBSs on cell division and migration were assessed by live video microscopy. Matrigel™ assays were used to determine the effects of these constructs on melanoma cells invasiveness.

Results:
We found that while AVBS1-2 promoted the formation of dynamic adhesion structures and membrane ruffles at the cell periphery, AVBS1-3, however, led to the formation of large ventral and peripheral Focal adhesions (FAs) containing the cytoskeletal linkers vinculin and talin3-5. These large FAs were extremely stable when compared to adhesion structures induced by AVBS1-2, and did not disassemble over extended period of times. Remarkably, and as opposed to control or AVBS1-2 transfected cells, AVBS1-3 induced FAs resisted treatment with the actin-relaxing drug blebbistatin, with a significant delay in the disappearance of the late FA marker VASP, suggesting that AVBS1-3 induced the formation of FAs in the absence of mechanosensing5. Consistently, while C2.7 myoblasts required plating on substrates with stiffness higher than 15 kPa to differentiate, AVBS1-3-transfected myoblasts formed multinucleated myotubes on 1.5 kPa stiffness substrates. Transfection of 1025Lu human melanoma cells with AVBS1-3 led to a drastic inhibition of cell division and motility, as well as of their capacity to invade Matrigel™.

Conclusion:
The results indicate that AVBS1-3 promote cell adhesion in the absence of mechanosensing and may represent a unique molecule to prevent cancer cells invasiveness.
References: (Times New Roman, font11)