The BRN2-derived peptide, L13S, inhibits metastasis of murine melanoma

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Introduction:
Melanoma is the most aggressive and deadliest form of skin cancer accounting for over 55,000 deaths/year worldwide. The transcription factor BRN2, under normal conditions, plays an essential role in cell differentiation and survival. In melanoma, however, BRN2 is overexpressed and known to regulate the expression of genes involved in cell migration and invasion thus being considered a key regulator of melanoma metastasis. Synthetic peptides rationally designed based on the POU domain of BRN2 have shown significant antitumor activity by competing with and blocking the binding of BRN2 on target promoter regions. This strategy represents a novel and promising therapeutic option to prevent the metastasis of melanoma.

Aim:
The present work aimed to determine, *in vitro* and *in vivo*, the anti-metastatic activity of the peptide L13S in a murine melanoma model.

Methodology:
To evaluate the inhibitory function of L13S in migration and invasion of B16F10-Nex2 cells, the Wound-Healing (scratch) assay and the use of Transwell inserts covered with Matrigel were respectively employed. To assess the internalization of the peptide, biotinylated L13S-treated B16F10-Nex2 cells were analyzed by confocal microscopy. To further investigate the mechanism of L13S internalization, a scratch assay in the presence of the macropinocytosis inhibitor amiloride was performed. To determine the therapeutic potential of L13S, C57BL/6 male mice (n=6) were inoculated intravenously with B16F10-Nex2 cells and subsequently treated with a 300 µg/day (i.p) dose regimen of freshly prepared peptide suspension. Ten days after the first treatment, the lungs were harvested and metastatic nodules manually counted. In parallel, a toxicity assay was carried out in mice treated with 1 mg/day of L13S for 3 consecutive days. Two days after the last treatment, the lungs, heart, liver, spleen, and kidneys were removed for histopathological analysis. The use of vertebrate animals was approved by the UMC Animal Care & Use Committee (CEUA; Protocols 005/2015 and 003/2018). Statistical analyses were performed using Student's *t-test*.

Results:
Confocal microscopy revealed that the L13S peptide was internalized by the cancer cells within 2 hours of treatment. Co-localization points of the peptide with actin fibers and the nuclei were detected suggesting that peptide is being transported through actin fibers to

the nuclei. *In vitro*, L13S inhibited cell migration by 10-fold in treated cells as compared to controls. Interestingly, its inhibitory activity was abrogated when cells were incubated with amiloride, suggesting that internalization of L13S occurs via macropinocytosis. L13S also inhibited cell invasion through Matrigel by approximately 50% when compared to controls. More importantly, L13S showed a protective effect in the experimental lung metastasis model, reducing the number of metastatic foci by 75% in the treated group as compared to the untreated control cohort (Untreated: 216, treated: 53, $p < 0.01$) with no off-target or tissue toxicity-related issues observed.

Conclusion:
Our data indicate that the peptide L13S is likely internalized by the cancer cells via macropinocytosis and transported to the nucleus through actin filaments. Our results also suggest that L13S can prevent melanoma metastasis by inhibiting both cell migration and invasion. Nevertheless, further studies are required to establish L13S as a novel therapeutic option for patients with melanoma.

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