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Characterization of c-MET in brain metastatic melanoma cell lines

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Introduction:

Although melanoma represent only 4% of all skin cancer, it accounts with the majority of the skin cancer deaths, mostly due to its ability to metastasize [1]. Indeed, survival rates are high when melanoma is detected in its early stages, but they decrease to less than 20% in advance melanomas that developed distal metastasis, due to its intrinsic biological aggressiveness and its typical resistance to medical therapy. Melanoma can metastasize in different organs, most common are skin, lung and brain. Approximately 7% of patients are diagnosed with melanoma brain metastases at first, and around 40-60% of metastatic patients develop brain metastasis during the course of their disease [2]. Recent evidences demonstrate that activation of c-Met pathway promotes brain metastasis in breast cancer [3].

Objectives:

We hypothesize that c-Met is involved in melanoma brain metastasis. For this purpose we have characterized the expression of c-MET in melanoma cell lines obtained from human melanoma brain metastasis and mouse cell lines derived from brain metastasis (B16-F10Br3). We postulate that c-MET give an advantage to melanoma cells for homing and establish brain metastasis. This process may be due to intrinsic or extrinsic properties (e.g. secretion of c-MET in exosomes and pre-metastatic niche formation). We analyzed c-MET expression in mouse and human melanoma cell lines, and the properties of these cell lines in 2D and 3D, we targeted c-MET by CRIPR-Cas9 system as a novel therapy to prevent melanoma metastasis.

Methodology:

Brain metastatic melanoma cell lines HM#86 and HM#19 cells were obtained from melanoma patients (Oslo University hospital). Mouse brain metastasis model B16-F10Br3 was obtained after 3 rounds of enrichment of brain metastasis from B16-F10 (Brain metastasis group, CNIO). We characterized c-MET expression in brain metastatic model in 2D and we generated 3D culture oncospheres from human cell lines characterizing oncospheres and exosomes derived from these cell lines by Western Blot and immunofluorescence.

Results:

We found that c-MET is highly expressed in metastatic human and mouse melanoma. While in mouse models brain metastatic cell line B16-F10Br3 showed 3 fold increase in c-MET and phospho-MET, in human brain metastatic models c-MET was increased in metastatic models regardless of metastatic site. Interestingly, in HM#86 cell line c-MET expression is increased in

in oncospheres when compared to 2D models of human. Moreover, high c-MET expression in cells correlates with the activation of AKT, ERK and MEK, which could indicate the involvement of these pathways in cell survival. Knock out of c-met in B16-F10Br3 by CRISPR-Cas9 system demonstrated that MET KO reduced the metastatic behavior of this cell line to lung and brain. Analysis of c-MET expression in brain melanoma metastasis showed a hyperexpression of this protein in a small group of metastasis (n=4).

Conclusions:

Our data demonstrate that c-Met is overexpressed in the brain metastatic mouse melanoma model B16-F10Br3, KO of c-Met in this model reduces lung and brain metastasis. In human melanoma cell lines c-MET is overexpressed in metastatic cell lines regardless of metastatic site. Interestingly, when brain metastatic cells HM86 were cultured as oncospheres, cMET expression was higher. Our data support the role of c-MET as a target to prevent melanoma metastasis and support its role in tumor cell survival. Future research will analyze the specific role of c-MET in brain metastasis as well as its secretion in exosomes and pre-metastatic niche formation in the brain

References:

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