Neutralizing antibody for S100A8/A9 soil sensing signal to prevent metastatic disease

Tiina E Kähkönen[1], Jussi M Halleen[1], Rie Kinoshita[2], Shigeru Morita[3], Kazumi Sagayama[4], Masakiyo Sakaguchi[2]

1) OncoBone Ventures Limited, 2) Department of Cell Biology, Faculty of Medicine, Dentistry and Pharmaceutical Science, Okayama University, 3) Center for Innovative Clinical Medicine, Okayama University Hospital , 4) The Organization for Research Strategy and Development, Okayama University

Contact tiina.kahkonen@oncobone.com for further information

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Introduction

Organotropism of cancer metastasis is driven by the 'seed and soil' theory where the soil (metastatic microenvironment) provides signals to attract the seeds (cancer cells) to grow on a certain location. S100A8/A9 is a 'soil sensing signal' that binds to receptors expressed by many cancer types (Table 1, Figure 1).

We aimed to understand the role of S100A8/A9 in melanoma, breast and lung cancer, to develop a S100A8/A9 neutralizing antibody, and to provide a proof-of-concept for the antibody for metastasis inhibition.

Table 1: S100A8/A9 receptors and their effects on cancer.

MCAM	Associated with metastasis and poor prognosis by modulating tumor cell migration
ALCAM	Involved in cancer progression, invasion, metastasis and recurrence: A novel cancer stem cell and tumor-specific prognostic marker
NPTN α and β	Induces EMT and potentiates lung metastasis
TLR-4	Through various immune mediators may cause immune response dysfunction, resulting in tumorigenesis
RAGE	Involved in carcinogenesis, tumor growth and inflammation
EMMPRIN	Plays a crucial role in cancer progression via several functions, including secretion of MMPs and enhanced angiogenesis

Figure 1: Expression of S100A8/A9 receptors in malignant and non-malignant tissues (from reference 1).

Materials and Methods

Cell lines were obtained from ATCC. Overexpression of S100A8/A9 receptors MCAM and NPTNβ was induced by a cDNA vector and MCAM expression was silenced by a siRNA. Recombinant S100A8/A9 protein was produced in-house. Migration/invasion assays were performed with Boyden Chamber method and colony formation assay with agarose gel cultures, and the results were quantitated by counting crystal violet -stained cells/colonies. mRNA expression was analyzed by qPCR. In mouse lung metastasis models, cancer cells were injected into tail vein, and lung metastases were monitored by X-ray Computed Tomography and quantitated by counting metastatic foci at endpoint. A chimeric S100A8/A9 antibody was produced in CHO-S cells by incorporating genomic sequences of heavy and light chains of a mouse monoclonal S100A8/A9 antibody to the Fab domain of human cDNA-encoded IgG2-Fc part.

Results

Key Findings in Breast Cancer

FIG 1: S100A8/A9 receptor MCAM is highly expressed in MDA-MB-231 invasive breast cancer cells
FIG 2: ETV4 is activated upon S100A8/A9 stimulation of MCAM resulting in increases migration
FIG 3: MCAM-mediated activation of ETV4 induces EMT via upregulation of ZEB1 and E-cadherin
FIG 4: Downregulation of ETV4 decreases tumor growth and lung metastasis

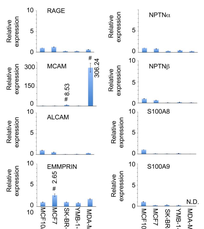

FIG 1. Relative expression of S100A8 and A9 and its receptors in breast cancer cells.

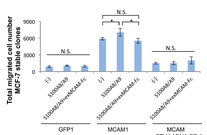

FIG 2. Number of migrated cells in control (GFP1), MCAM overexpressing (MCAM1) and MCAM overexpressing ETV4 non-functional (MCAM + ETV4 ΔDNA-BD1) cells in response to 100 ng/ml extracellular S100A8/A9 in the presence or absence of 1000 ng/ml exMCAM-Fc decoy recombinant protein.

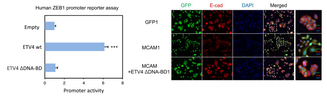

FIG 3. ZEB1 reporter assay in control ETV4 overexpressing or ETV4 ΔDNA-BD cells (left). E-cadherin (E-cad) IF in control (GFP1), MCAM overexpressing (MCAM1) and MCAM overexpressing + ETV4 ΔDNA-BD1 cells (right).

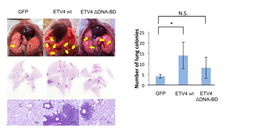

FIG 4. Histological appearance and quantitated amount of lung metastases in ETV4 overexpressing and ETV4 ΔDNA-BD breast cancer cells.

Key Findings in Melanoma

FIG 1: MCAM is highly expressed in malignant melanoma and especially in the invasive front of the tumor
FIG 2: MCAM is highly expressed in invasive melanoma cells (WM-266-4) and silencing of MCAM decreases invasion and the cells lose sensitivity to S100A8/A9 stimulation
FIG 3: ETV4 was identified to mediate increased migration induced by S100A8/A9 stimulation of MCAM
FIG 4: Overexpression of MCAM and ETV4 increases formation of melanoma lung metastases whereas tumors with dominant negative ETV4 produce less lung metastases

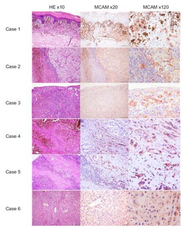

FIG 1. IHC of MCAM in malignant melanoma in 6 patients.

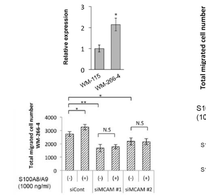

FIG 2. Relative expression of MCAM in non-invasive WM-115 and invasive WM-266-4 melanoma cell lines (upper). Effects of MCAM silencing in WM-266-4 cells on migration with or without stimulation of S100A8/A9 (lower).

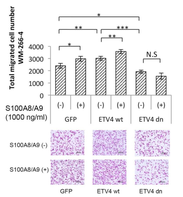

FIG 3. Effects of ETV4 overexpression or ETV4 dn on melanoma migration with or without S100A8/A9 stimulation.

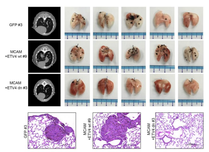

FIG 4. Formation of lung metastases from control (GFP), MCAM + ETV4 overexpressing (ETV4 wt) and MCAM + ETV4 dn melanoma cells.

Key Findings in Lung Cancer

FIG 1: NPTNβ is highly expressed in lung cancer cell lines and the expression is higher in lung cancer biopsy samples than in non-malignant tissues
FIG 2: NPTNβ overexpressing cells stimulated with S100A8/A9 have increased colony formation, migration and invasion
FIG 3: Binding of S100A8/A9 to NPTNβ mediates activation of NFIA and NFIB and activates SPDEF transcription factor, causing increased migration and invasion
FIG 4: Activation of NPTNβ and NFIAIB pathway increases the number of metastatic loci in lungs, showing higher number of proliferating cells and cytokeratin 8 staining

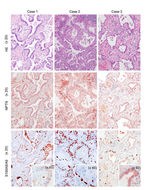

FIG 1. IHC of S100A8/A9 and NPTNβ in lung cancer biopsy samples.

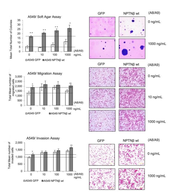

FIG 2. S100A8/A9 increases colony formation, migration and invasion of control and NPTNβ overexpressing A549 lung cancer cells.

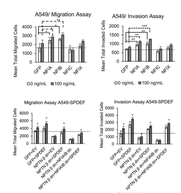

FIG 3. Migration and invasion of A549 lung cancer cells transfected with control or NF-family proteins (upper panel), and A549 cells transfected with SPDEF, NPTNβ and/or NFIAIB treated with vehicle or S100A8/A8 (lower panel).

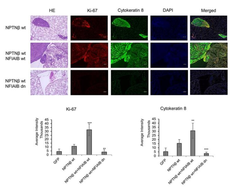

FIG 4. IHC and analysis of Ki-67 and cytokeratin 8 levels in NPTNβ and NFIAIB expressing or dominant negative lung metastasis.

S100A8/A9 Antibody Development

FIG 1: Series of antibodies for S100A8/A9 were prepared with different affinity profiles to S100A8 and A9 separately, and S100A8/A9 heterodimer
FIG 2: The developed antibodies differentially inhibited migration of cancer cells and secretion of cytokines including TNFα, IL-6 and IL-8 when stimulated with S100A8/A9
FIG 3: The developed antibodies differentially inhibited the formation of melanoma lung metastases

Based on the results antibody #45 was selected for further development

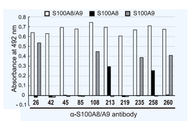

FIG 1. Antibody affinity to S100A8, A9 or S100A8/A9 heterodimer in ELISA assay.

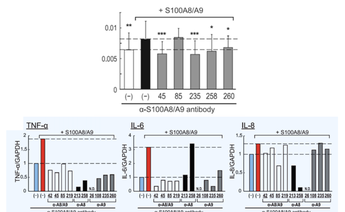

FIG 2. Ability of the developed antibodies to inhibit migration when stimulated with S100A8/A9 protein (upper). Ability of the developed antibodies to regulate secretion of TNFα, IL-6 and IL-8 cytokines (lower panel).

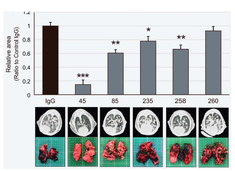

FIG 3. Ability of the developed antibodies to prevent formation of lung metastases in a syngeneic B16-BL6 mouse melanoma model in a 2-week study.

Graphical summary

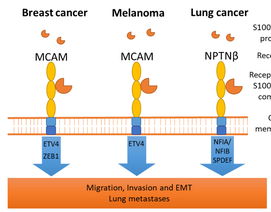

Figure 2: Summary of identified cellular mechanisms in breast cancer, melanoma and lung cancer leading to increased migration, invasion, EMT and finally formation of lung metastases due to stimulation by S100A8/A9.

The developed antibody #45 binds effectively S100A8/A9 heterodimer leading to effective inhibition of migration and production of pro-inflammatory cytokines, and prevention of lung metastasis in breast cancer and melanoma animal models.

Conclusions

These results demonstrate a role for S100A8/A9 and its receptors in metastasis of melanoma, breast and lung cancer. A neutralizing antibody for S100A8/A9 prevents formation of lung metastases, suggesting pharmacological inhibition of S100A8/A9 as a potential option for preventing metastatic disease.

Future directions

The neutralizing S100A8/A9 antibody #45 will be studied in cancer metastasis models to provide proof-of-concept for inhibiting other metastases such as bone metastases, followed by regulatory safety studies.

Acknowledgements

We would like to acknowledge Okayama University personnel who have contributed to the research.

About OncoBone Ventures:
OncoBone Ventures is a biotech startup company developing novel therapies for cancer patients with bone metastases. OncoBone Ventures has partnered with Okayama University to co-develop the S100A8/A9 antibody towards clinical development.

Abbreviations:
ALCAM = Activated Leukocyte Cell Adhesion Molecule; ATCC = American Type Culture Collection; DAPI = 4',6-diamidino-2-phenylindole (nuclear stain); DN = Dominant Negative; EMMPRIN = Extracellular Matrix Metalloproteinase Inducer; EMT = Epithelial-to-Mesenchymal Transition; ETV = ETS Variant Transcription Factor; GFP = Green Fluorescent Protein; IHC = Immunohistochemistry; MCAM = Melanoma Cell Adhesion Molecule; MCAM +ETV4 ΔDNA-BD1 = MCAM overexpressing ETV4 non-functional cells; MMP = Matrix Metalloproteinase; NF = Nuclear Factor; NFIAIB = Nuclear Factor 1 A and B; NPTN = Neuroplastin; TLR = Toll-like receptor; RAGE = Receptor for Advanced Glycation Endproducts; SPDEF = SAM Pointed Domain Containing ETS Transcription Factor; ZEB1 = Zinc Finger E-Box Binding Homeobox 1.

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