

ONLINE DATA SUPPLEMENT

Aminopeptidase N/CD13 as a Potential Therapeutic Target in Malignant Pleural

Mesothelioma

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SUPPLEMENTAL METHODS

Angiogenesis Antibody Array Analysis

EHMES-10 (2.5×10^5) cells were seeded in 6-well plates and incubated with MT95-4 (40 $\mu\text{g/mL}$) or human control IgG for 24 hours. Cells were rinsed with phosphate-buffered saline and dissolved in lysis buffer (NP-40), and the protein concentration in the cell lysates was determined and normalized using a BCA assay kit (Pierce Biotechnology, Rockford, IL, USA) to ensure that the same amount of total protein was used for each assay. Human angiogenesis antibody array analysis (R&D Systems, Minneapolis, MN, USA) was performed according to the manufacturer's instructions. Briefly, the samples were placed on the membrane and incubated at 4°C overnight, followed by incubation with a biotinylated detection antibody for 2 hours at room temperature. Horseradish peroxidase-streptavidin solution was added to the membrane and incubated for 2 hours at room temperature. The densities of individual spots were visualized using a chemiluminescence detection system (WSE-6100; ATTO, Tokyo, Japan) and measured using ImageSaver6 (ATTO).

Table S1, related to Figure 6**Array map of Angiogenesis Antibody Array Analysis****Array 1**

	A	B	C	D	E	F	G	H
1	Positive Control	Positive Control	Negative Control	Negative Control	Angiogenin	EGF	ENA-78 (CXCL5)	bFGF
2								
3	Growth Factor	IFN- γ	IGF-1	IL-6	IL-8 (CXCL-8)	Leptin	MCP-1 (CCL2)	PDGF-BB
4								
5	PLGF	RANTES (CCL5)	TGF- β 1	TIMP-1	TIMP-2	TPO	VEGF-A	VEGF-D
6								
7	BLANK	BLANK	BLANK	BLANK	BLANK	BLANK	Negative Control 1	Positive Control
8								

Array 2

	A	B	C	D	E	F	G	H
1	Positive Control	Positive Control	Negative Control	Negative Control	ANGPT1	ANGPT2	Angiostatin	Endostatin
2								
3	GCSF	GM-CSF	I-309 (CCL1)	IL-10	IL-1 α	IL-1 β	IL-2	IL-4
4								
5	I-TAC (CXCL11)	MCP-3 (CCL7)	MCP-4 (CCL13)	MMP-1	MMP-9	PECAM-1 (CD31)	Tie-2	TNF α
6								
7	uPAR	VEGF R2	VEGF R3	BLANK	BLANK	BLANK	BLANK	Positive Control
8								