Essential Role of Fibroblast Growth Factor Receptor 2 (FGFR2) in Tumorigenesis of Human Cancers Harboring *FGFR2* Amplification Demonstrated by a Functional Blocking Antibody

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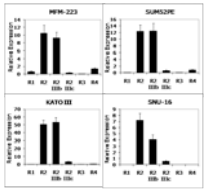
Introduction

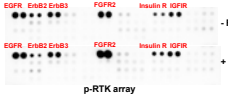
Fibroblast growth factors (FGFs) play important roles in regulating many fundamental biological processes including embryogenesis, tissue homeostasis, metabolism, angiogenesis, and wound healing. Dysregulated FGF signaling has been implicated in the pathogenesis of human cancers. We generated monoclonal antibodies (mAbs) against the extracellular ligand binding domain of fibroblast growth factor receptor 2 (FGFR2) to address the role of FGFR2 in tumorigenesis and to explore the potential of FGFR2 as a novel therapeutic target. Human gastric and breast cancer cell lines harboring *FGFR2* amplification predominantly express the IIIb-isoform of FGFR2. Therefore, we used an FGFR2-IIIb specific antibody, AV369b, to investigate the importance of FGFR2 signaling in such cell lines *in vitro* and *in vivo*.

FGFR copy number and expression in human cancer cell lines

Cell line	Cancer Type	Copy Number			
		FGFR1	FGFR2	FGFR3	FGFR4
MFM-223	Breast	6.7	286.5	2.4	2.4
SUM52PE	Breast	6.6	113.2	3.2	4
KATO III	Gastric	2.3	137.0	3.6	2.6
SNU-16	Gastric	1.7	271	1.3	1.6
HEC-1-A	Endometrial	1.1	1.1	1.8	1.8
AN3 CA	Endometrial	2	2.9	1.8	2.9
MFE-296	Endometrial	2	1.8	2.4	2.3
MFE-280	Endometrial	2.9	1.8	3.5	2.8
MFE-319	Endometrial	1.7	1.6	2.1	1.8
Ess-1	Endometrial	1.8	2.1	1.9	1.9
NCI-H2122	Lung	2.5	2.9	2.2	2
NCI-H1703	Lung	11.7	3	3.9	4
NCI-H661	Lung	3.7	2.1	3.1	2.8
NCI-H1437	Lung	2.4	4	3	2.7
NCI-H1975	Lung	1.5	2.6	2.9	1.8
NCI-H69	Lung	2	3	3	2.8

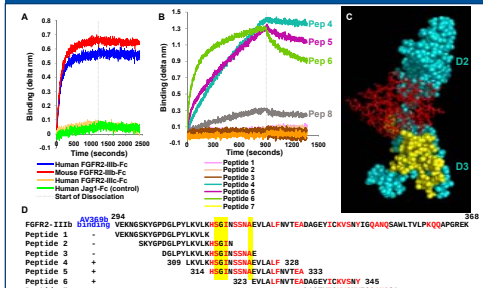


Tyrosine phosphorylation status of 42 receptor tyrosine kinases (RTKs) in SNU-16 cells ± FGF7



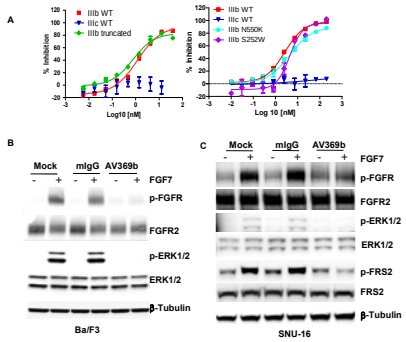
SNU-16 cells were either untreated or treated with FGF7 (30 ng/mL) and heparin (20ug/ml) for 10 min, and protein lysates were analyzed with a phospho-RTK array (R&D Systems) which can simultaneously detect the relative phosphorylation of 42 different RTKs.

Epitope mapping of AV369b



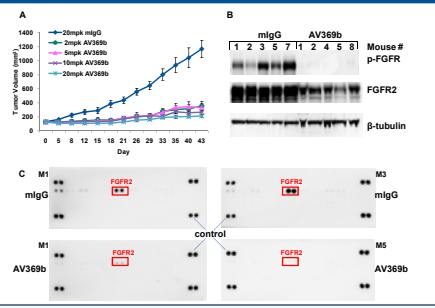
A. Specific binding of AV369b to human and mouse FGFR2-IIIb determined by Octet biosensors (ForteBio).
B. Epitope mapping using overlapping peptides.
C. AV369b binding peptides (yellow) mapped to structure of FGFR2 IIIb (blue) in complex with FGF10 (red).
D. Peptide sequences used for epitope mapping. The residues in human FGFR2-IIIb that differ from FGFR2-IIIc are in red. The AAs involved in binding to FGF10 are highlighted in yellow.

AV369b suppresses FGFR2-IIIb-driven proliferation and downstream signaling



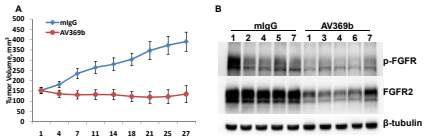
A. AV369b suppresses cell proliferation driven by the WT or the C-terminally truncated variant of FGFR2-IIIb, or mutant variants of FGFR2-IIIb. AV369b mAb was added to FDCP-1 cells expressing FGFR2-IIIb, -IIIc, a C-terminally truncated IIIb variant, or the S252W and N550K mutant variants in the presence of FGF1 (8ng/ml) and heparin (5ug/ml).
B. AV369b suppresses FGF7-induced FGFR2 tyrosine phosphorylation and ERK1/2 phosphorylation in Ba/F3 cells overexpressing FGFR2-IIIb.
C. AV369b blocks FGF7-induced FGFR2 and FRS2 tyrosine phosphorylation and ERK1/2 phosphorylation in SNU-16 cells.

AV369b inhibits *in vivo* growth of SNU16 xenografts



A. SCID mice inoculated with SNU-16 cells received mIgG control at 20 mg/kg, or AV369b at 2, 5, 10 or 20 mg/kg by intra-peritoneal injection twice weekly.
B. Analysis of total FGFR2 and phospho-FGFR in SNU-16 tumors treated with either the control IgG or AV369b. β-tubulin was used as a loading control.
C. Tyrosine phosphorylation status of 42 RTKs in individual tumors collected at the end of study from mice treated with mIgG or AV369b.

Treatment of MFM-223 xenografts with AV369b results in tumor stasis



A.. Nude mice bearing MFM-223 cells received either mIgG or AV369b at 20 mg/kg by intra-peritoneal injection twice weekly.
B Analysis of total FGFR2 and phospho-FGFR in MFM-223 tumors treated with either the control IgG or AV369b.

Summary

1. **AV369b potently suppressed ligand-induced phosphorylation of FGFR2-IIIb and downstream signaling *in vitro*.**
2. **The administration of AV369b in mice significantly inhibited the growth of *FGFR2*-amplified human cancer xenografts.**
3. **These findings support an essential role of FGFR2 in the initiation and/or maintenance of human cancers harboring *FGFR2* amplification. Cancer patients with activated/amplified FGFR2 signaling could potentially benefit from therapeutic intervention with FGFR2-targeting antibodies.**

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