New Monoclonal Antibody Targeting on Basic Fibroblast Growth Factor (bFGF) against Melanoma, Lung Cancer and Breast Cancer

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Abstract code: 15307

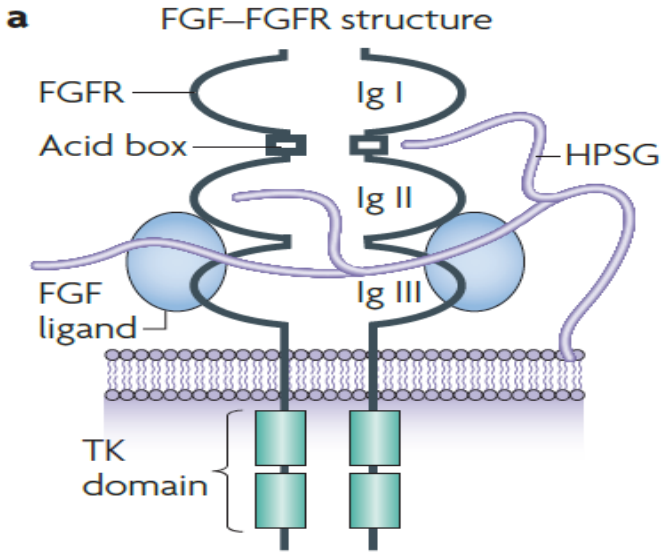
Disclosure of Interest: None Declared

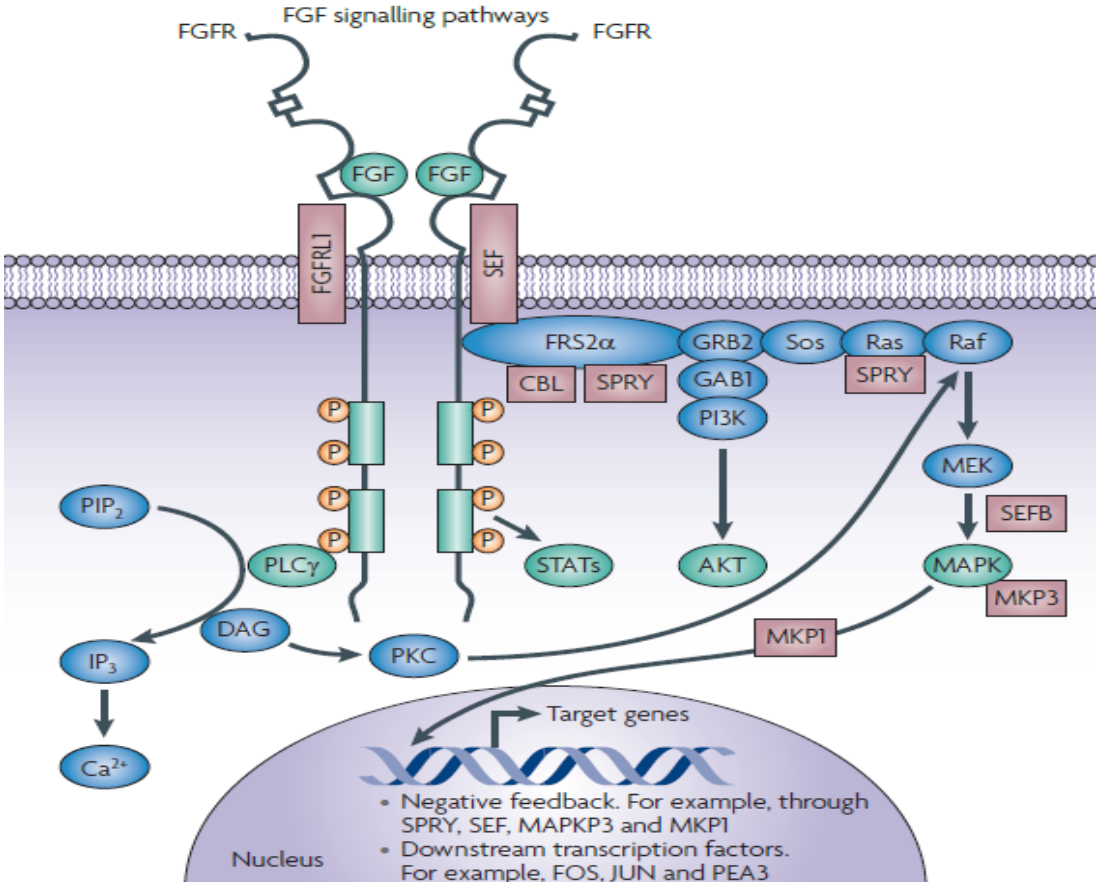
Cancer Targets and inhibitor agents

- Better understanding of cancer biology leads to strategic target discovery
- Effective discovery of molecular target therapy
- Demonstrated efficacy in preclinical research

bFGF/FGFR

signaling network





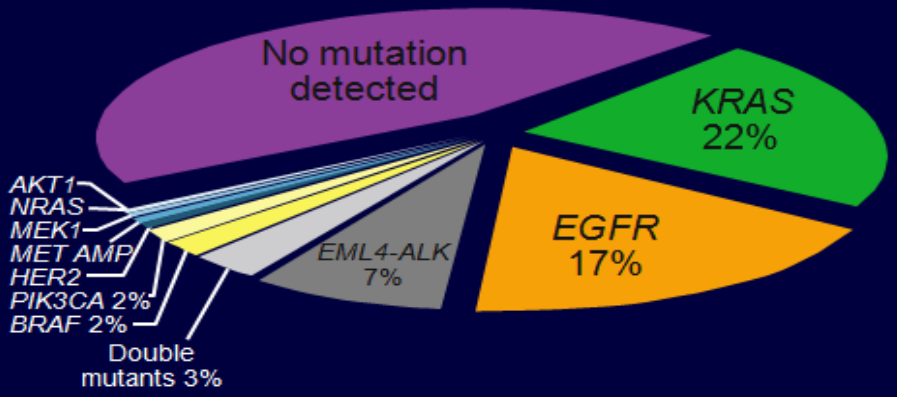
Turner et al . Nat Rev Cancer, 2010, 10(2): 116-129.

Fibroblast Growth Factor (FGF)

- **A family of 22 members, FGF variously binds FGF receptor (FGFR) isoforms.**
- **Different tumors express FGF, correlated with poor prognosis.**
- **FGF pathway activation is a potent driver in lung cancer.**
- **~20% primary lung caner with mutations/amplification in FGFR.**
- **Autocrine activation of FGFR , contributed to EGFR inhibitor insensitivity.**

Potential "Druggable" Molecular Targets?

Lung Cancer Molecular Consortium Lung Adenocarcinomas



Mutations found in 54% (280/516)

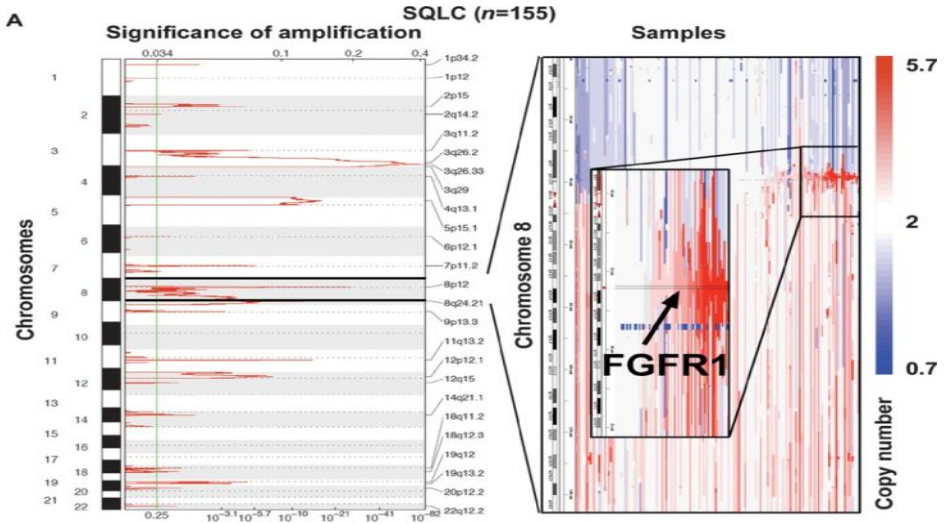
Emerging "Druggable" Targets in NSCLC-Squamous Subtype

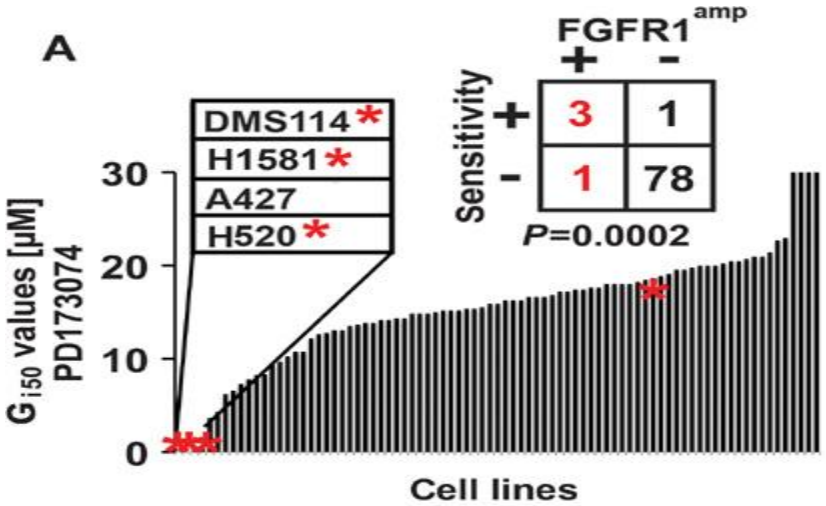
Gene	Event Type	Frequency, %
FGFR1	Amplification	20-25
FGFR2	Mutation	5
PIK3CA	Mutation	9
PTEN	Mutation deletion	18
CCND1	Amplification	8
CDKN2A	Deletion/mutation	45
PDGFRA	Amplification mutation	9
EGFR	Amplification	10
MCL1	Amplification	10
BRAF	Mutation	3
DDR2	Mutation	4
ERBB2	Amplification	2

Kris MG, et al. ASCO 2011. CRA7506. Johnson BE, et al. IASLC WCLC 2011. Abstract O16.01

Hammerman P, et al. IASLC WCLC 2011. Abstract PRS.1

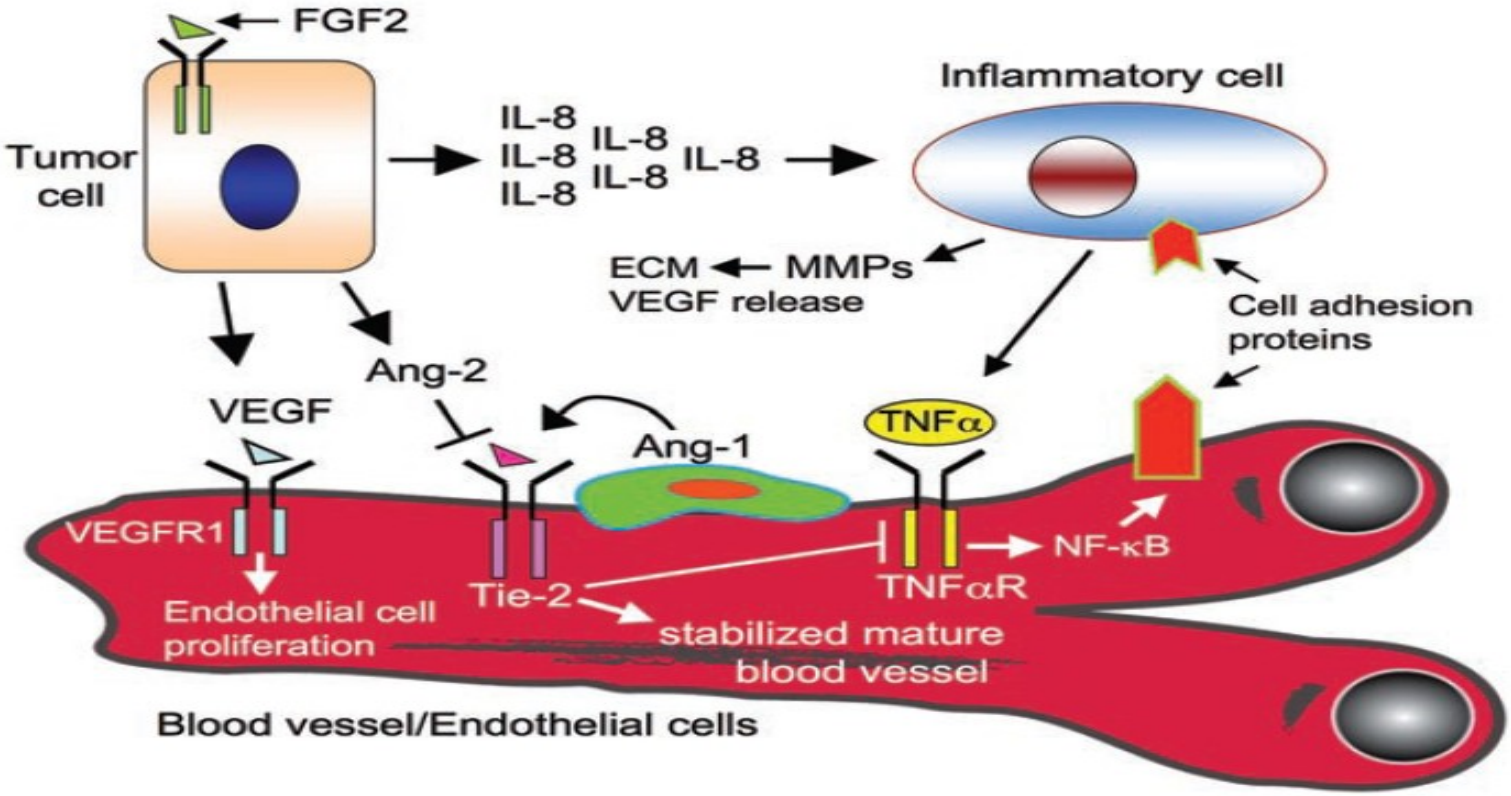
FGFR1 Amplified and Sensitivity to FGFR Inhibition in Squamous NSCLC





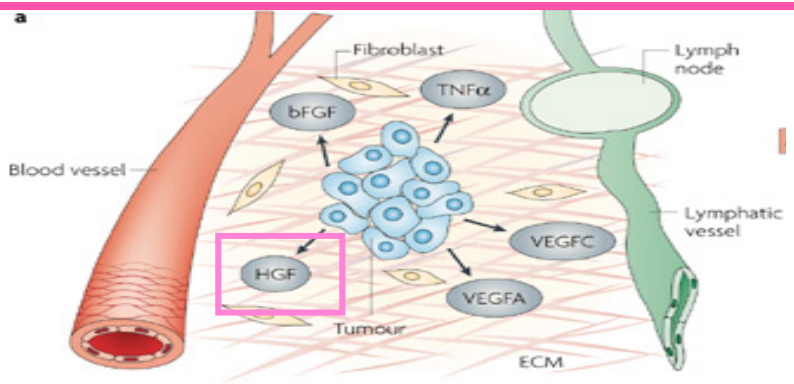
From Weiss J, et al. Sci Transl Med. 2010;2:62ra93.

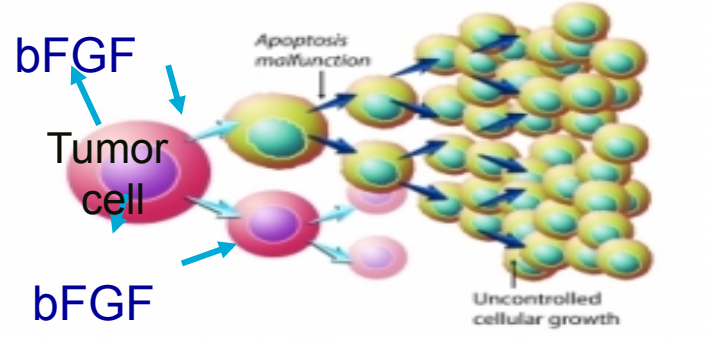
Activation of bFGF in tumor cells

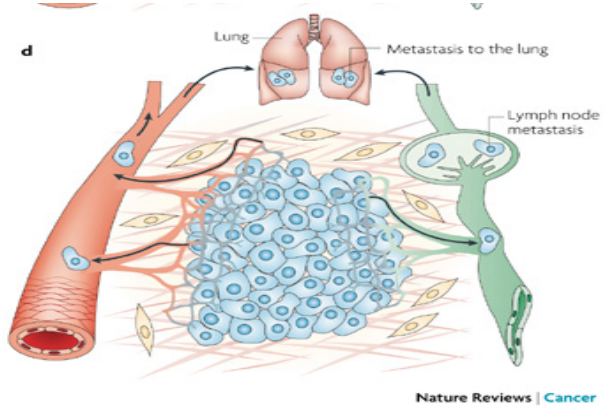


Acevedo et al. Cell Cycle, 2009, 8(4): 580-588.

basic fibroblast growth factor (bFGF)







bFGF is an important molecule that involved with proliferation, apoptosis, angiogenesis, invasion and metastasis.

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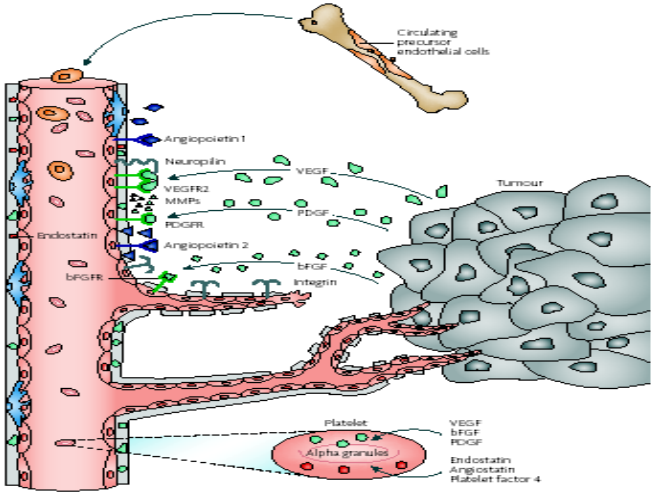
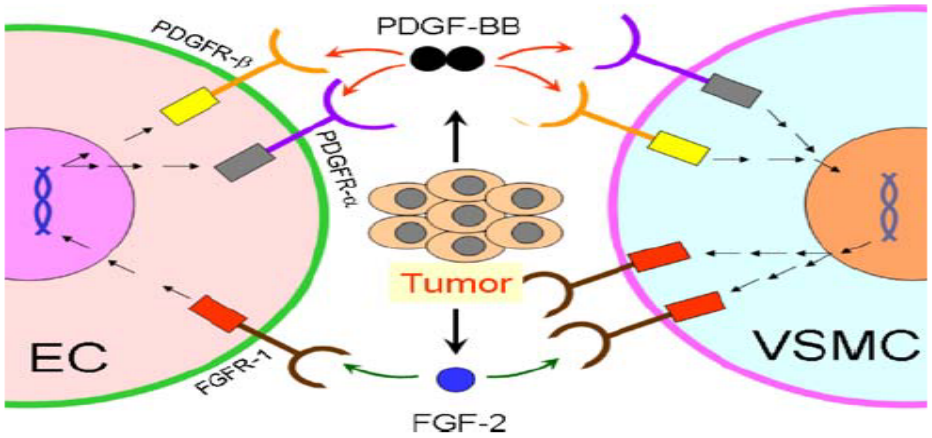
Good tumor marker target

Nature Reviews Drug Discovery, 2007, 6(4): 273-286

The interaction of bFGF with other pro-angiogenic growth factors





bFGF collaborates with VEGF, PDGF-induced tumor angiogenesis and promote tumor growth .

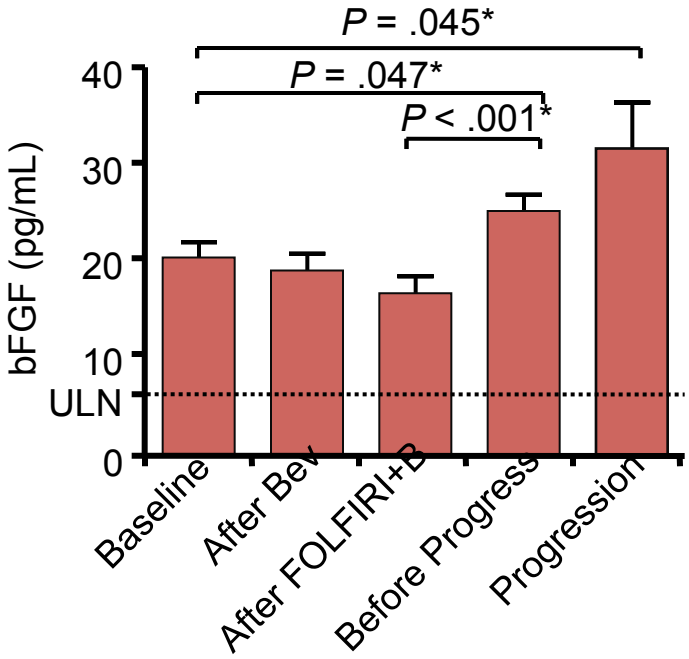
bFGF stimulates endothelial cell to secrete VEGF, which can promote tumor angiogenesis.

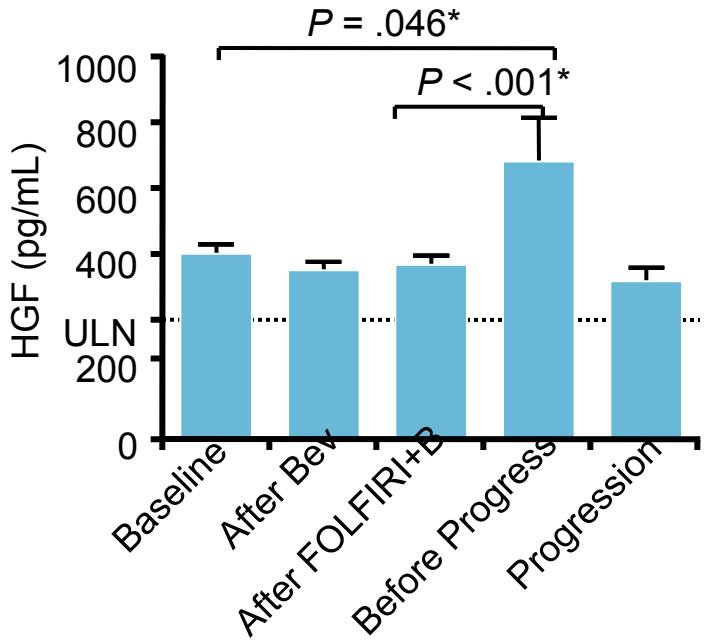
bFGF combines with FGFR, which promote the expression of PDGFRαand PDGFRβ .

PDGF-BB up-regulates expression of FGFR-1 and VSMCs, and enhance the sensitivity of bFGF.

J Mol Med，2008，86（7）：785-789

Elevations in bFGF and HGF Develop Prior to Progression

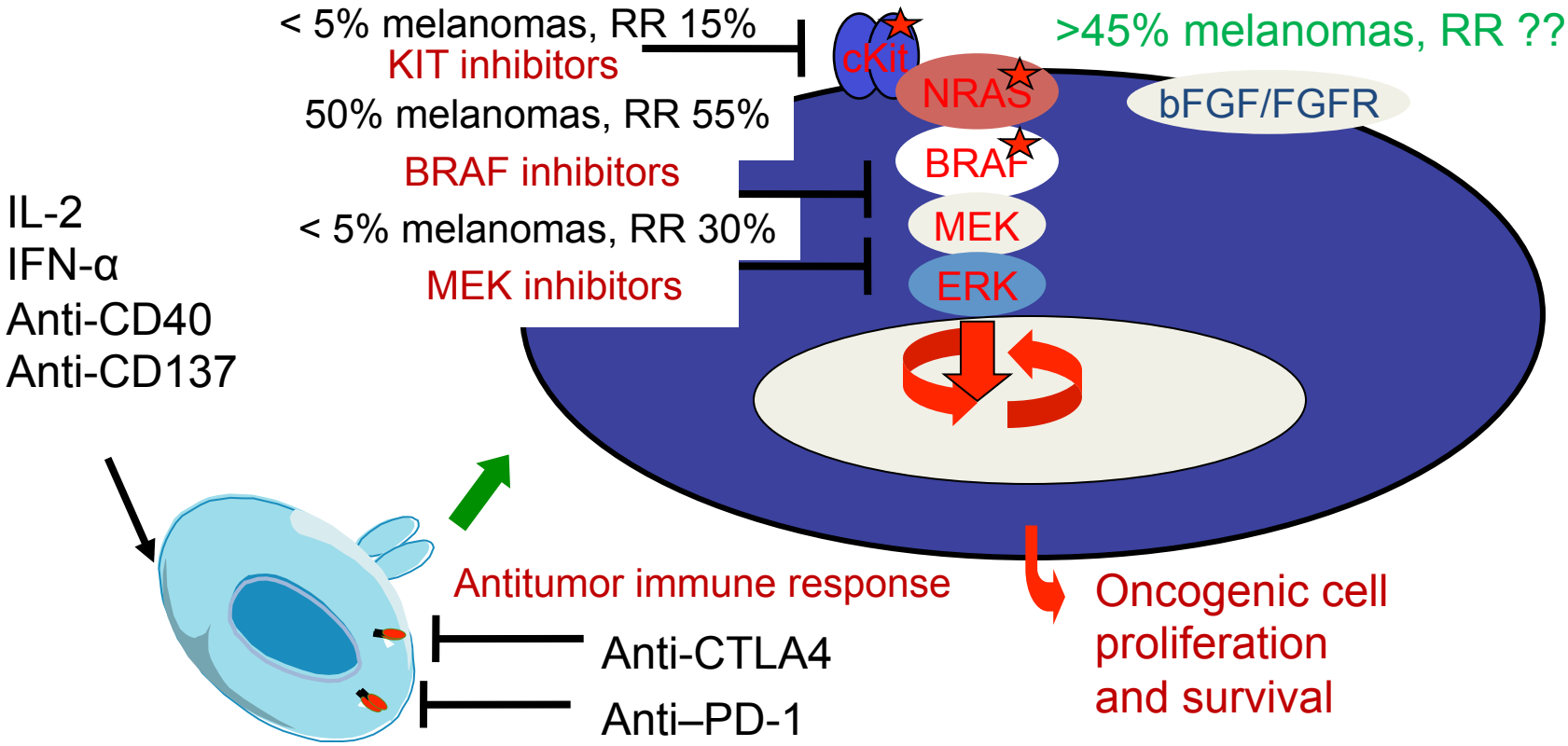




*Significantly different after multiple comparison correction, with significance defined by local false discovery rate q < 0.05.

Kopetz S, et al. J Clin Oncol. 2010;28:453-459.

Target therapy and Immunotherapy for Melanoma: Response

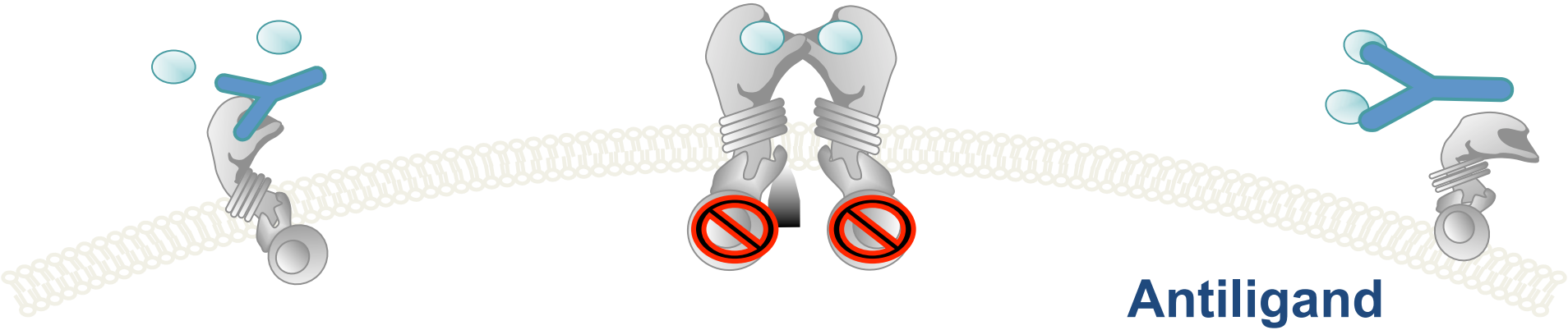


Importance of Targeting FGF/FGFR Pathway

- Involved in angiogenesis, proliferation, chemotherapy resistance, and metastatic progression.
- Deregulation by mutations, FGFR gene amplifications.
- Associated with resistance to VEGF inhibitors.
- Associated with acquired resistance to bevacizumab in colorectal cancer.

Grose R, et al. Cytokine Growth Factor Rev. 2005;16:179-186. Casanovas O, et al. Cancer Cell. 2005;8:299-309. Kopetz S, et al. J Clin Oncol. 2010;28:453-459.

bFGF/FGFR1: Targeted Approaches



Antireceptor blocking antibodies

Tyrosine kinase inhibitors

Antiligand blocking antibodies

Anti-bFGF mAb

Table 2 | **Current status of FGF and FGFR-targeting therapies***

Drug name	Company	Range of activity or target	Clinical development	Refs
Small molecular tyrosine kinase inhibitors				
SU5402	*In vitro* reagent	Selective FGFR inhibitor (now superseded by availability of PD173074)	NA	75
PD173074	*In vitro* reagent	Selective FGFR inhibitor	NA	184
TKI258	Novartis	FGFR, PDGFR and VEGFR inhibitor	Phase II	185
BIBF 1120	Boehringer Ingelheim	FGFR, PDGFR and VEGFR inhibitor	Phase III	186
BMS-582,664 (Brivanib)	Bristol-Myers Squibb	FGFR and VEGFR inhibitor	Phase II	187
E7080	Eisai	FGFR, PDGFR and VEGFR inhibitor	Phase I	188
TSU-68	Taiho Pharmaceutical	FGFR, PDGFR and VEGFR inhibitor	Phase I/II	189
FGFR antibodies and FGF ligand traps				
IMC-A1	ImClone	FGFR1-IIIc-specific antibody	NA	156
PRO-001	ProChon Biotech	FGFR3-specific blocking antibody	NA	84
R3Mab	Genentech	FGFR3-specific antibody	NA	155
1A6	Genentech	FGF19-specific antibody	NA	104
FP-1039	Five Prime Therapeutics	FGF ligand trap (multiple FGFs)	Phase I	157
FGF ligand for mucosal chemoprotection				
Palifermin (Kepivance)	Biovitrum AB	Recombinant FGF7 (activates FGFR2-IIIb)	Licensed	190

FGF, fibroblast growth factor; FGFR, FGF receptor; NA, not applicable; PDGFR, platelet-derived growth factor receptor; VEGFR vascular endothelial growth factor receptor.*Several pharmaceutical companies have highly potent and selective FGFR inhibitors in preclinical development.

Turner et al. Nat Rev Cancer, 2010, 10(2): 116-129.

- **Focused on bFGF key role, bFGF-targeted therapy research and new anti-bFGF mAb which neutralizes bFGF, blocks its ability to activate FGFR1 in treating solid tumors.**

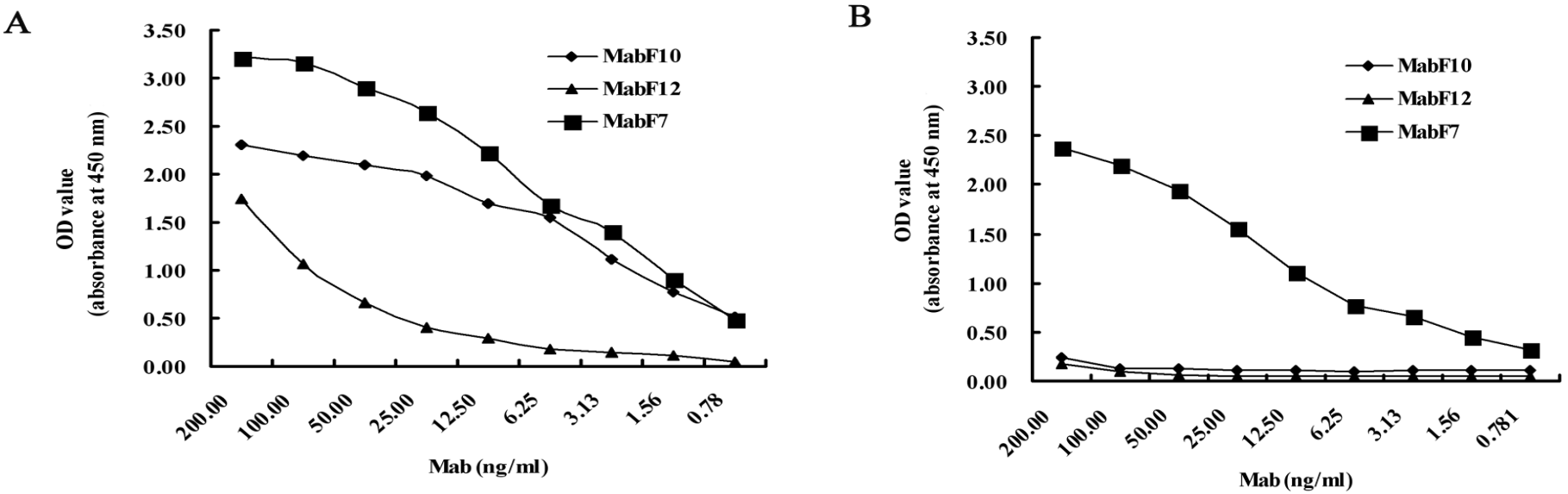
- **The antitumor, antiangiogenesis, antimetastatic and reversal of multidrug resistance (MDR) activities of anti-bFGF mAb could be investigated.**

- **The effectiveness in combination with standard therapeutic schedules and pharmacokinetic profile of anti-bFGF mAb will be established.**

Characterization of anti-bFGF mAbs

Mab	Subtype	KD（M）	The cross -reactivity with aFGF	The cross -reactivity with VEGF	Epitope
MabF7	IgG1	10^{-9}	no	no	linear epitope
MabF10	IgG1	3.8×10^{-7}	no	no	space epitope
MabF12	IgG1	4.5×10^{-7}	no	no	space epitope

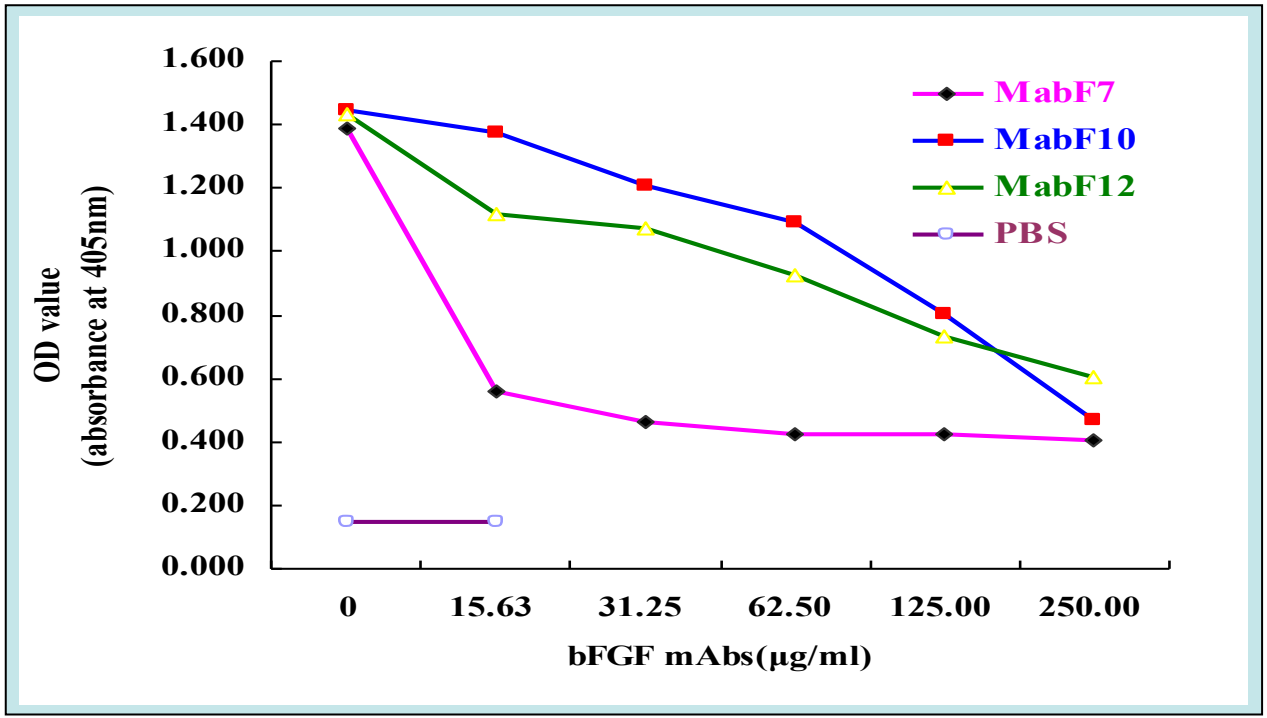
board package of natural and denatured bFGF assay with indirect ELISA method



Determination of the continuous or discontinuous epitopes recognized by anti-bFGF mAbs

A: Natural bFGF package board

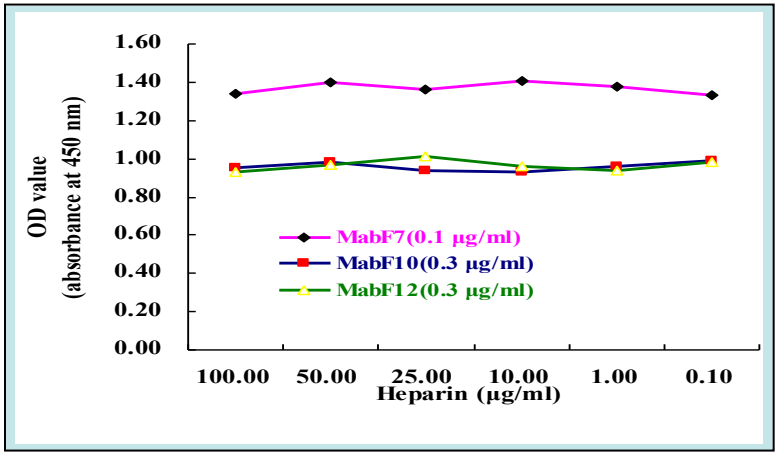
B: thermal denaturation bFGF package board



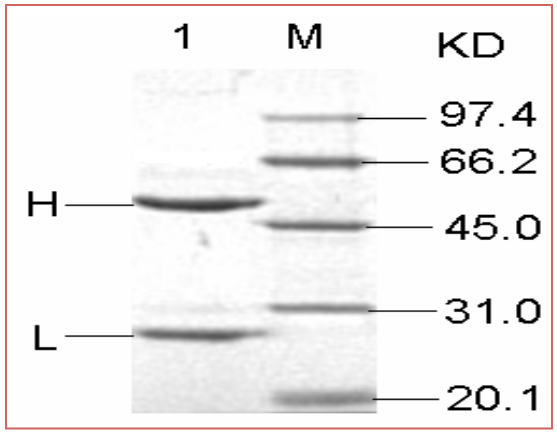
anti-bFGF mAb MabF7, MabF10 inhibit bFGF binding with its affinity recetor FGFR-1βIIIC.

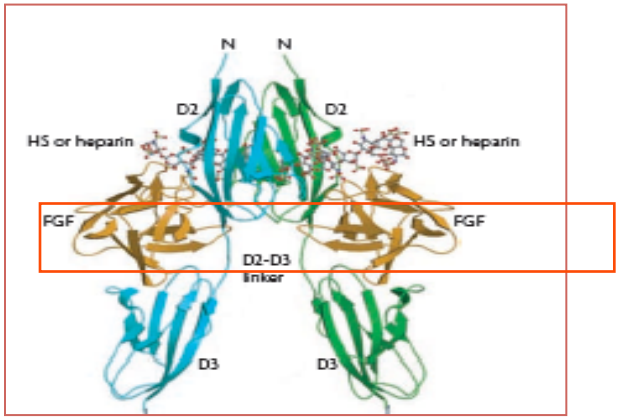
IC50 is 7.5 μg/ml, 65 μg/ml and 50 μg/ml respectively

Inhibition of binding of FGFR1β(IIIc)/Fc to immobilized bFGF by anti-bFGF mAbs



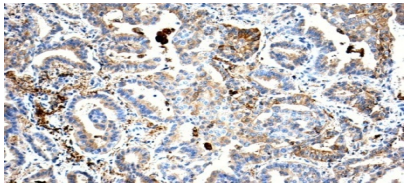
Effect of binding of heparin to immobilized bFGF by anti-bFGF mAbs.



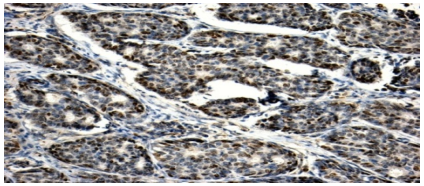


There is no effect when bFGF binding to heparin, this showed that the three monoclonal antibodies can recognize receptor binding epitope region.

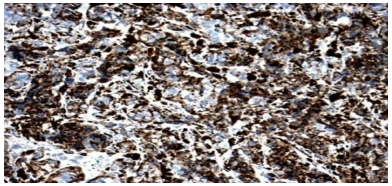
Purified anti-bFGF mAb ,H: heavy chain of antibody, L : light of the antibody.



Lung cancer

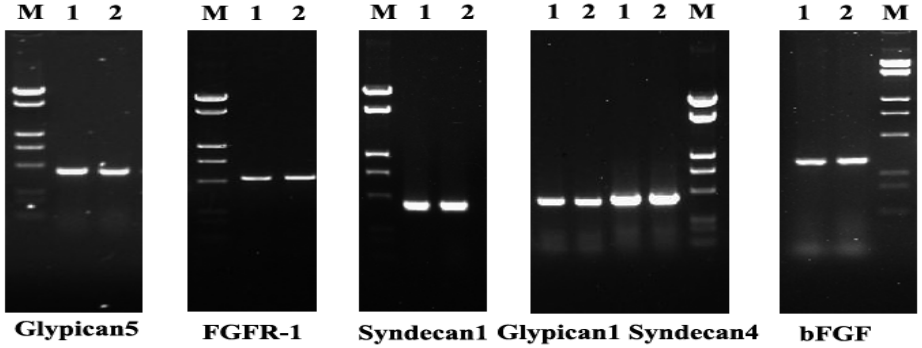


Breast cancer



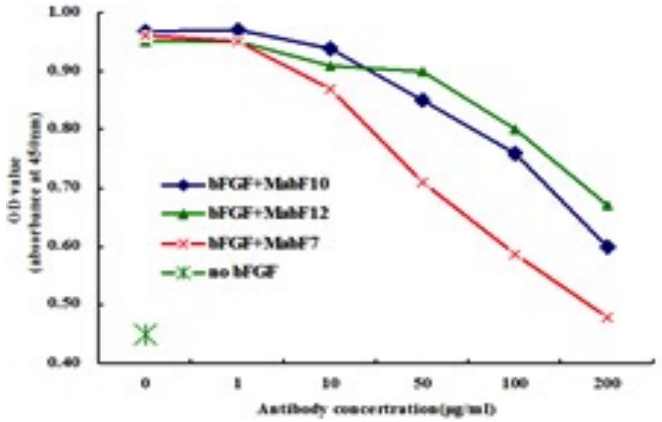
Melanoma

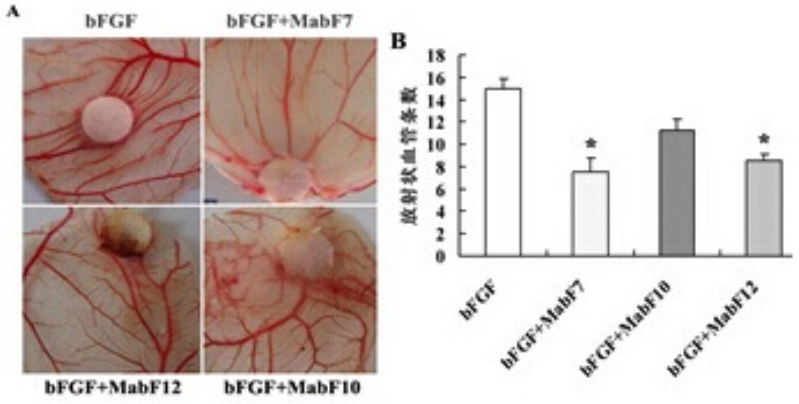
bFGF expression in different tumors(100×)



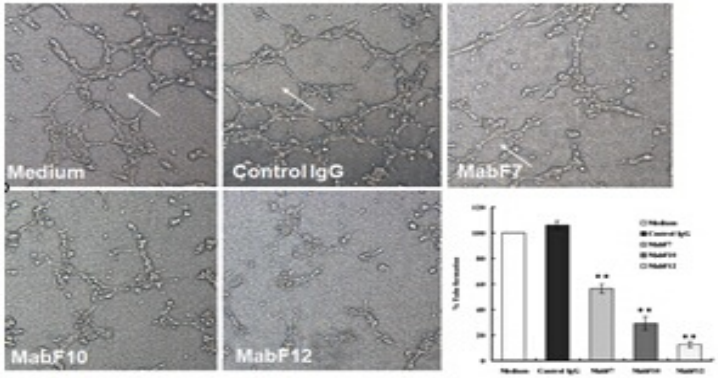
RT-PCR detected the expression of bFGF / FGFR and related moleculars

Expression of bFGF/FGFR， Glypican1，Glypican5，Syndecan1, Syndecan4 in B16 and B16F10, M: DL3000；1：B16; 2：B16F10



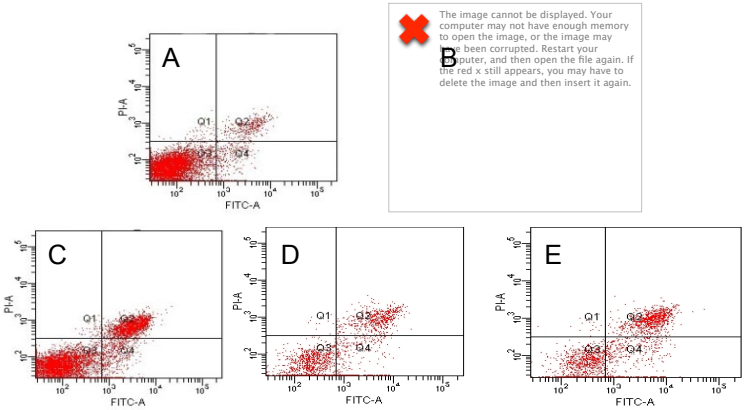


The experiment of suppression chorioallantoic membrane angiogenesis

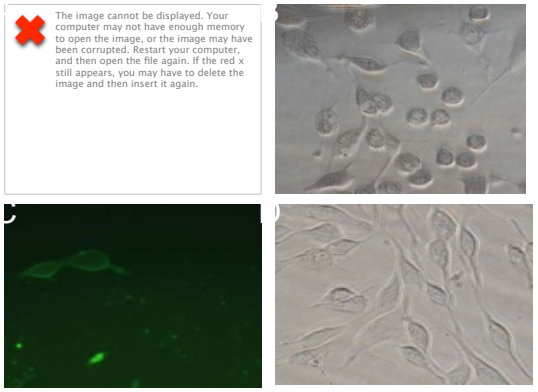


The effects of bFGF monoclonal antibody against the vascular endothelial cells

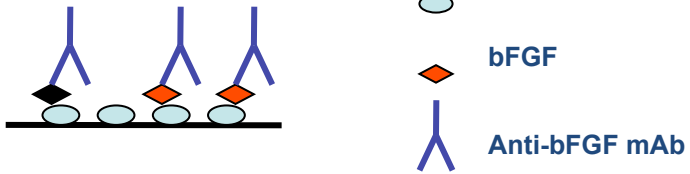
Anti-bFGF mAb inhibiting tumor growth and angiogenesis



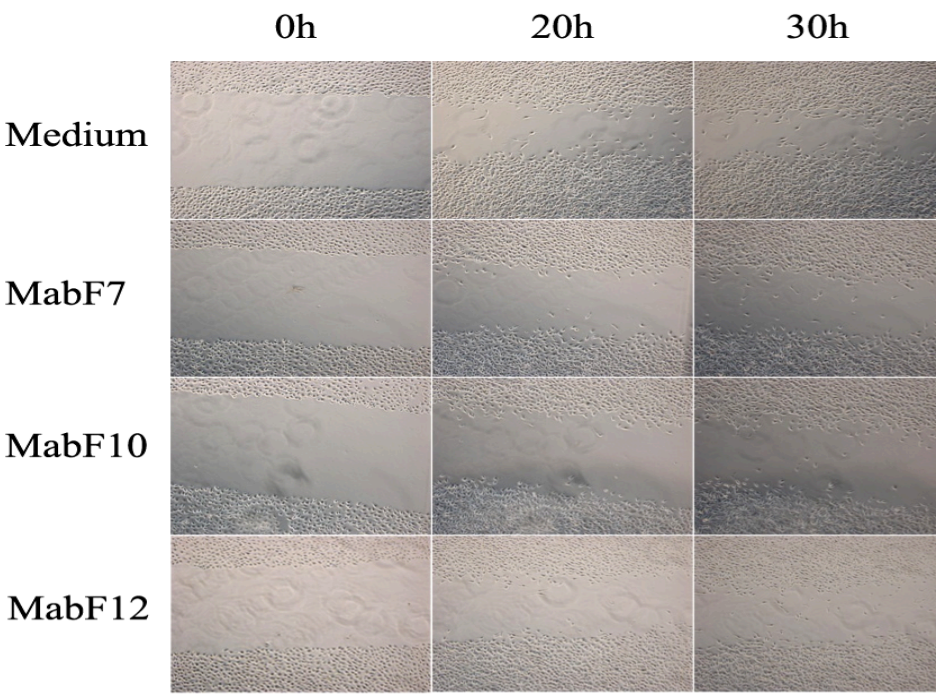
Flow cytometry results of anti-bFGF mAb inducing B16 cell apoptosis



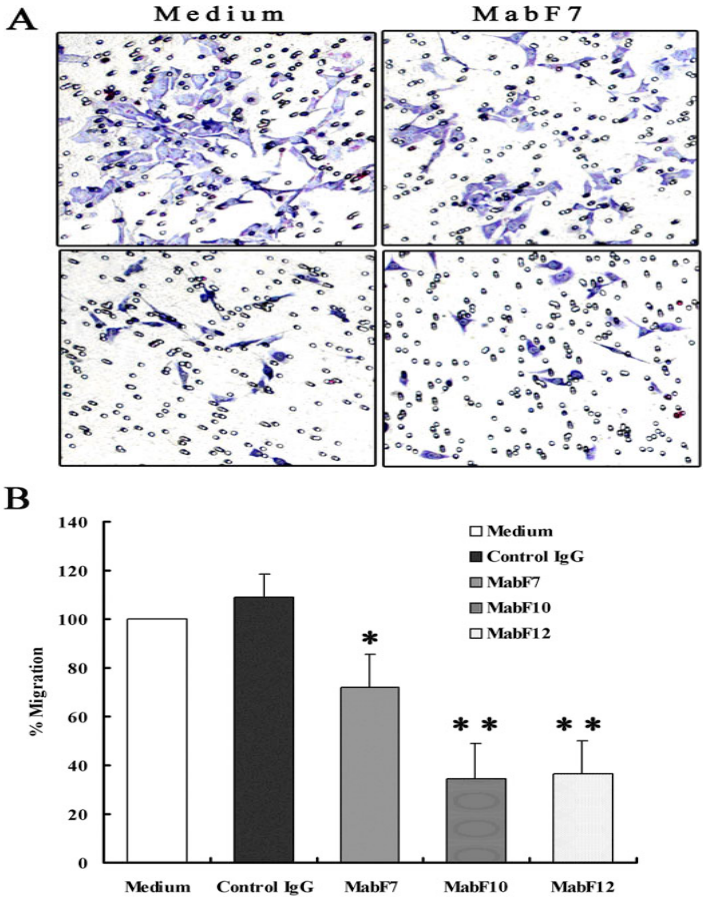
Microscopic observations of anti-bFGF mAb inducing apoptosis



Anti-bFGF mAbs induce the apoptosis of melanoma *in vitro*



Inhibition of B16F10 migration by anti-bFGF mAbs in vitro （scratch test)

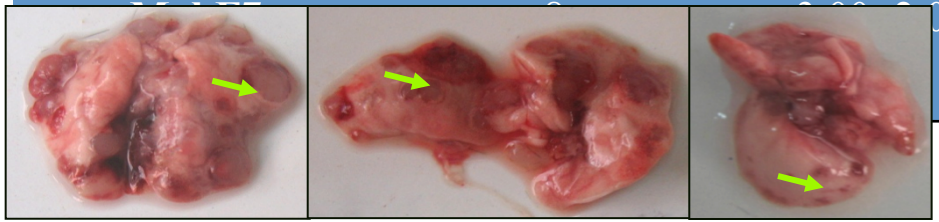


Inhibition of melanoma cells migration by anti-bFGF mAbs (transwell)

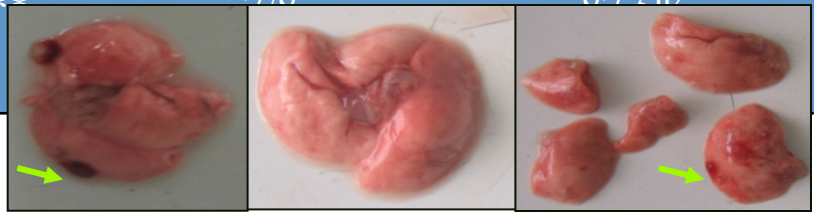
Inhibition rate:
75.59%

Inhibition of lung cancer metastasis by MabF7

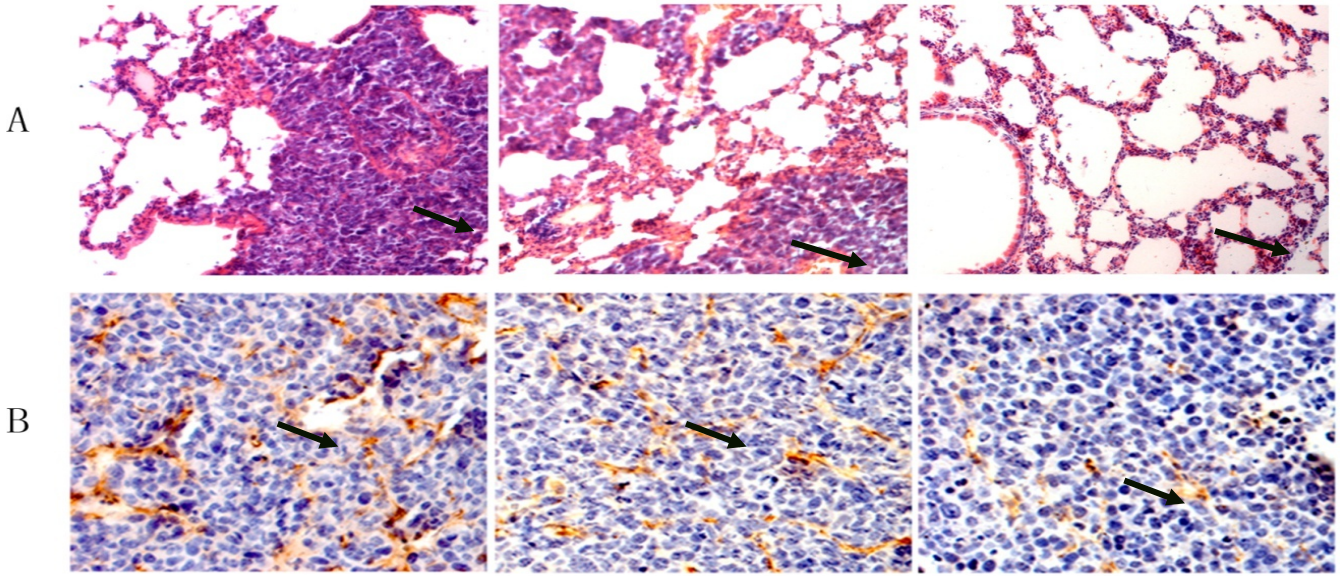
group	Number (only)	Nodules (±S D)	Transfer Rate	
			Transfer number / total number	Percentage
PBS	8	12.29±2.37	8/8	100%
control **IgG**	8	11.80±3.59	8/8	100%
		09**		



(A) PBS



(B) MabF7

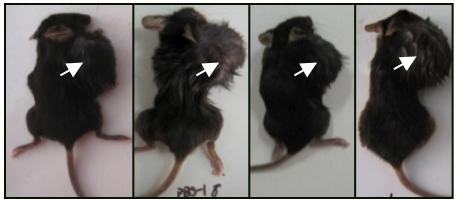


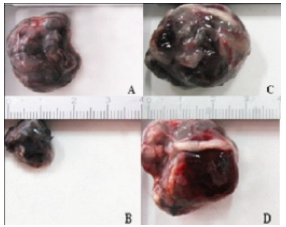
MVD inhibition rate：49.43%

Inhibition of metastasis and MVD in lung cancer by MabF7

A: Metastatic nodules of mouse lung (black arrow，×200)；

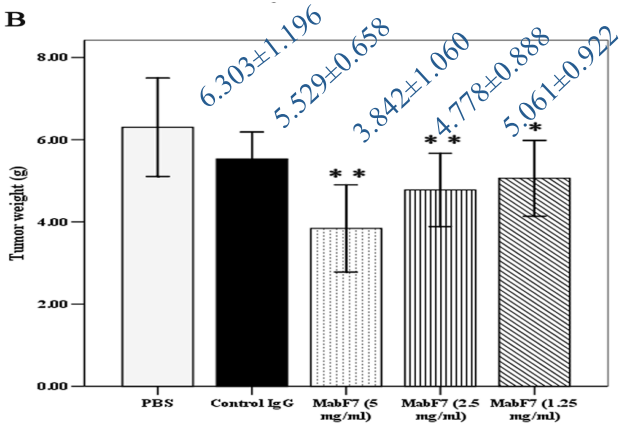
B：MVD in tumor tissue（black arrow，×200）

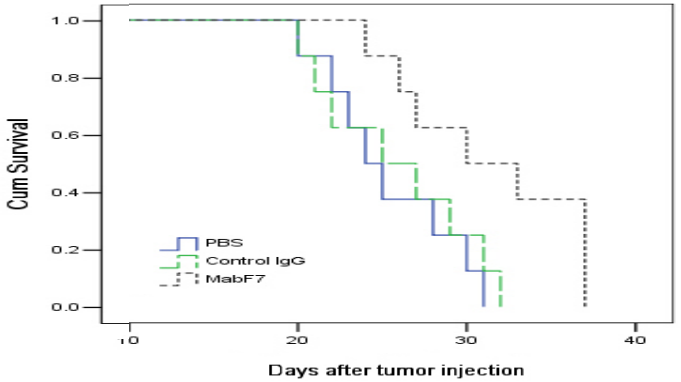




MabF7(5mg/ml)

control





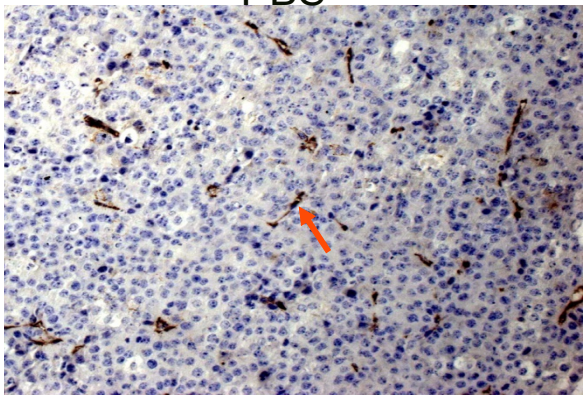
The median survival time of PBS group, Control IgG and MabF7 is 24, 26, 32 days respectively.

Survival curves of mice

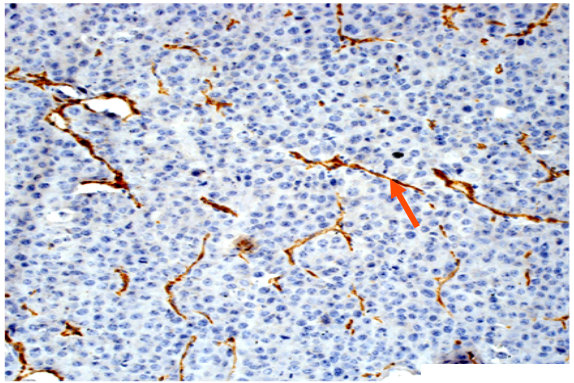
The volume tumor inhibition rate of MabF7 was 46.40%, 23.24%, 19.89% respectively,

Inhibition of B16 melanoma tumor growth by anti-bFGF mAbs *in vivo*

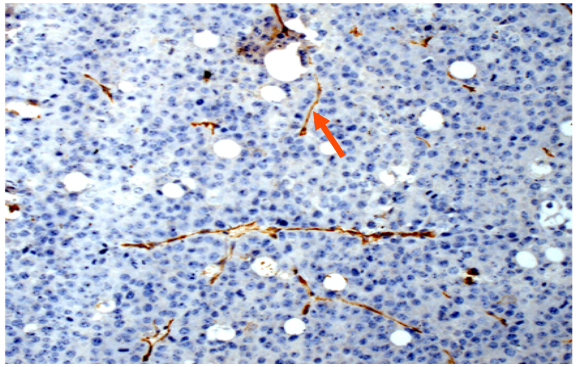
PBS



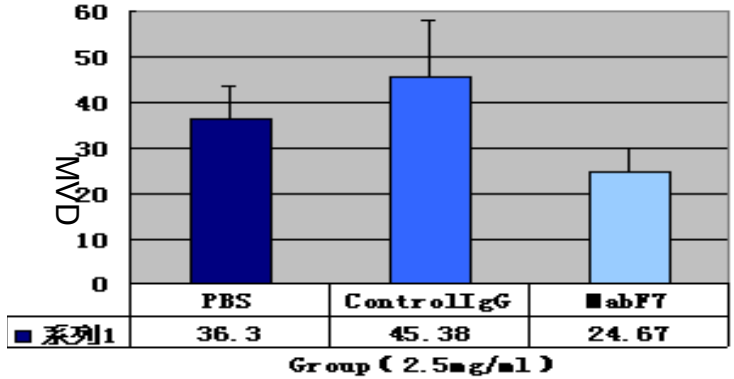
Control IgG



MabF7



Blood vessel density in tumor tissues from treated mice:CD31 immunohistochemical staining



Inhibition effects of anti-bFGF mAb combined with radiotherapy on B16 melanoma in

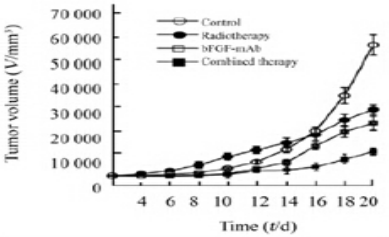


Fig. 1 Radiotherapy combined with bFGF-mAb inhibited growth of B16-transpalnted tumors

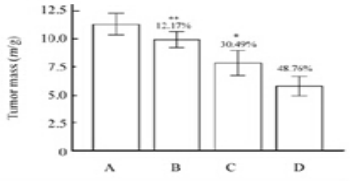


Fig. 2 Radiotherapy combined with bFGF-mAb therapy inhibited proliferation of B16-transpalnted tumors

A: Control group; B: Radiotherapy group; C: bFGF-mAb group; D: Combined group

$^{*}P<0.05$, $^{**}P<0.01$ *vs* D group

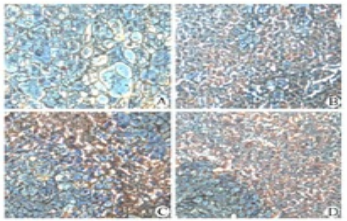


Fig. 3 bFGF-mAb combined with radiotherapy promoted apoptosis of B16-transpalnted tumor cells (×400)

A: Control group; B: Radiotherapy group; C: bFGF-mAb group; D: Combined group

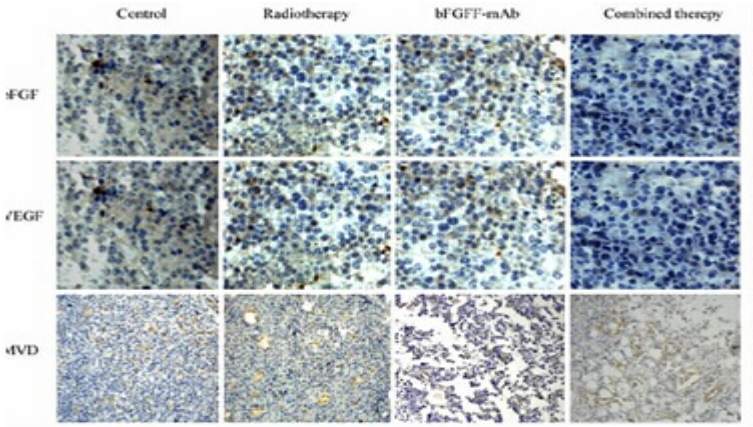


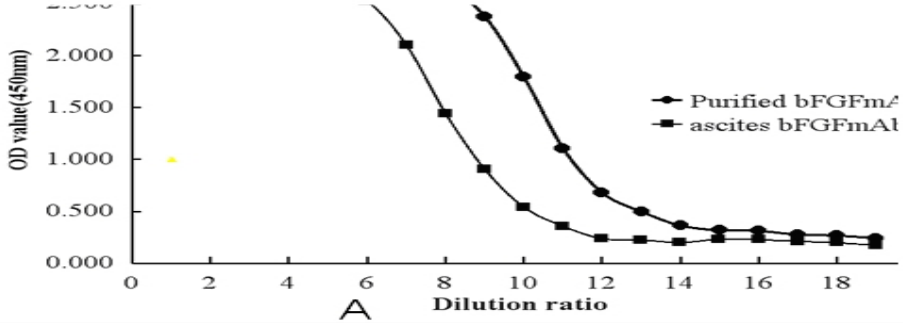
Fig. 4 bFGF-mAb combined with radiotherapy inhibited bFGF, VEGF expressions and tumor angiogenesis of B16-transpalnted tumors (S-P, ×200)

Tab. 1 bFGF and VEGF expression rates and MVD of B16-transpalnted tumors in different groups

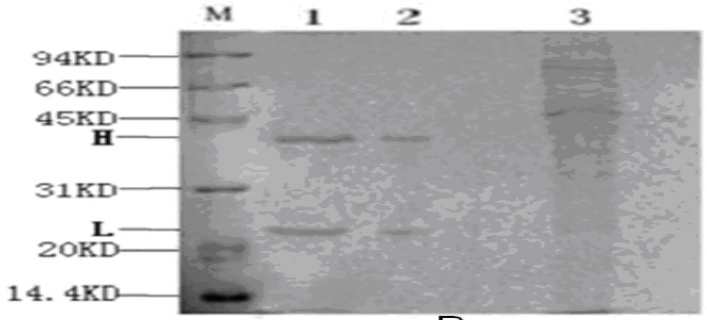
Group	bFGF(%)	VEGF(%)	MVD
Conrtol	$67.3\pm9.4^{**}$	$57.2\pm7.1^{**}$	$68.5\pm17.2^{**}$
Radiotherapy	$34.3\pm6.3^{**\triangle\triangle}$	$28.5\pm4.7^{*\triangle\triangle}$	$42.8\pm6.2^{**\triangle\triangle}$
bFGF-mAb	$20.6\pm4.6^{*\triangle\triangle}$	$42.3\pm5.1^{**\triangle}$	$58.5\pm13.4^{**\triangle}$
Combined	11.4 ± 2.4	17.5 ± 3.4	24.7 ± 6.5

$^{*}P<0.05$, $^{**}P<0.01$ *vs* combined group; $^{\triangle}P<0.05$, $^{\triangle\triangle}P<0.01$ *vs* control group

Pharmacokinetics study of anti-bFGF mAb



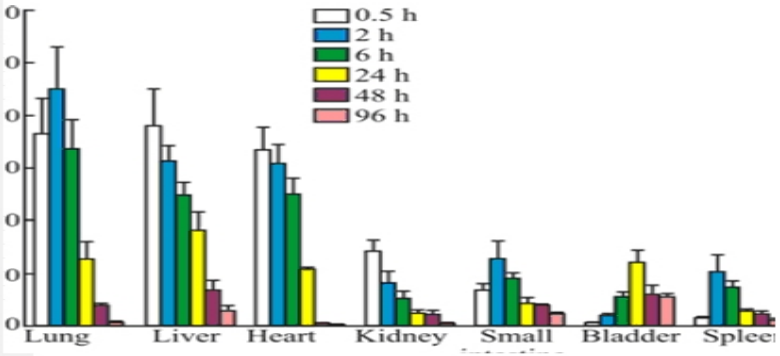
A



B

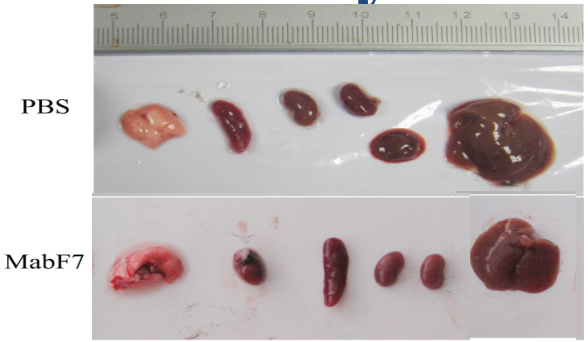
Parameter	Value/mg · kg^{-1}		
	0.5	1.5	5
$T_{\frac{1}{2}}\alpha$/h	0.10 ±0.03	0.20 ±0.02	0.135 ±0.05
$T_{\frac{1}{2}}\beta$/h	1.05 ±0.11	1.24 ±0.09	1.84 ±0.07
$T_{\frac{1}{2}}\gamma$/h	81.57 ±4.93	86.79 ±7.25	90.02 ±5.29
C_{5min}/ g · L^{-1}	8.16 ±1.23	25.38 ±3.46	82.04 ±3.37
CL/ml · h^{-1} · g^{-1}	3.83 ±0.24	2.15 ±0.09	2.9 ±0.15
$AUC_{(0-144)}$/g · h · L^{-1}	96 ±5.34	485 ±70.61	1 212 ±110.87
$AUC_{(0-\infty)}$/g · h · L^{-1}	119 ±6.83	685 ±80.52	1 745 ±130.59

B

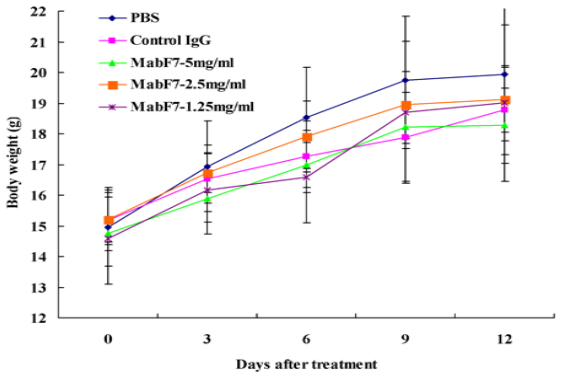


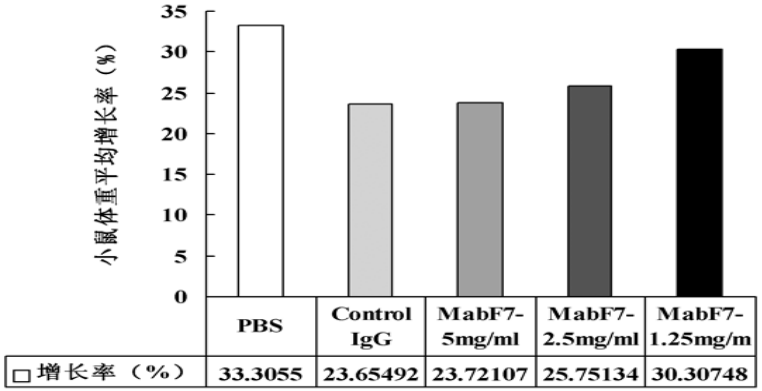
D

The toxicity of anti-bFGF mAb in mice

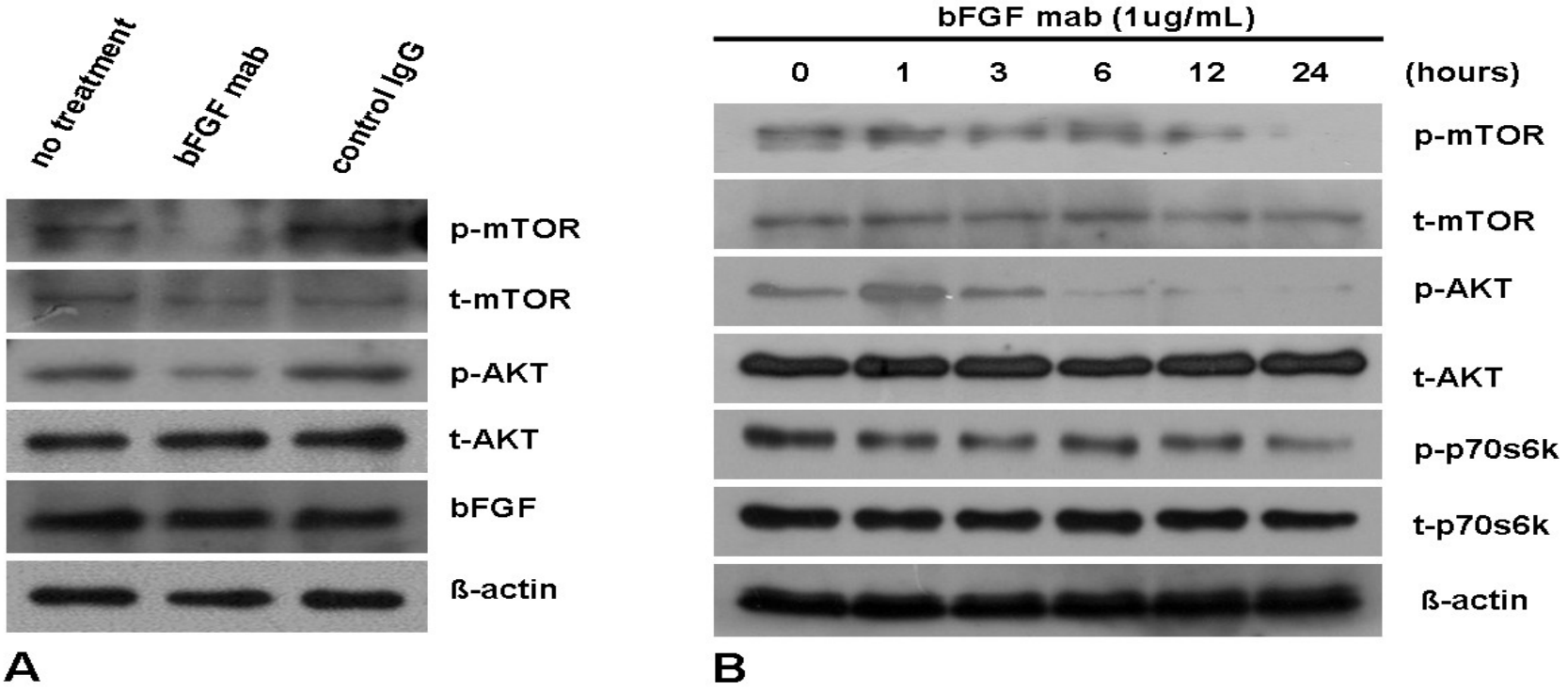


Organ of mice treated with MabF7
(no organ damage)

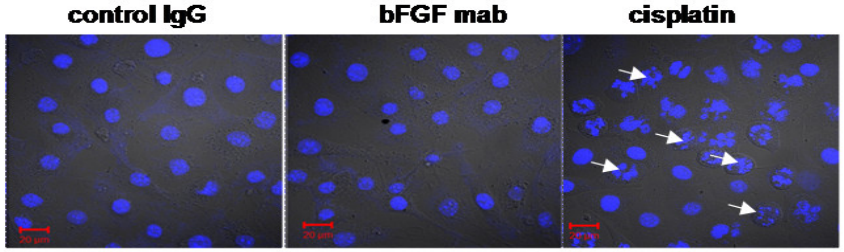




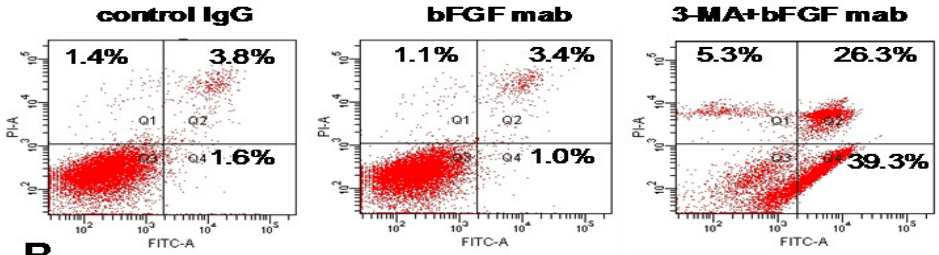
Effect of anti-bFGF mAbs on mice by body weight



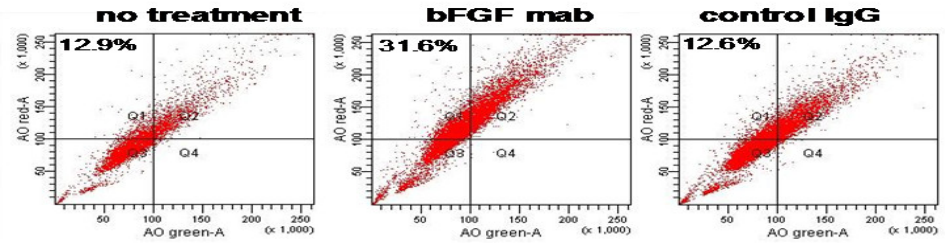
The AKT-mTOR signaling pathway of **anti-**bFGF **mAb** inhibiting B16 cells



A



B



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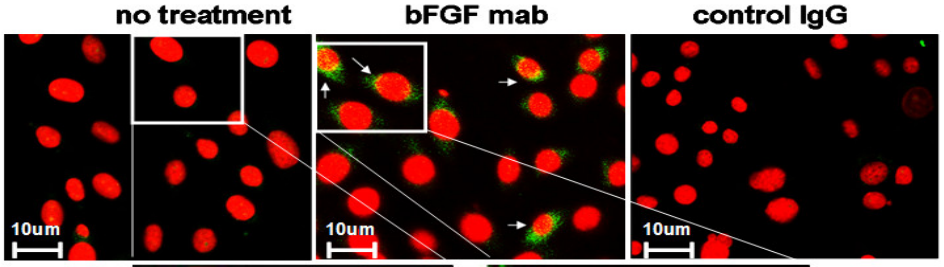

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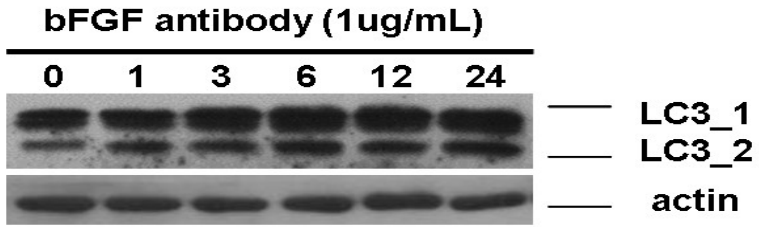
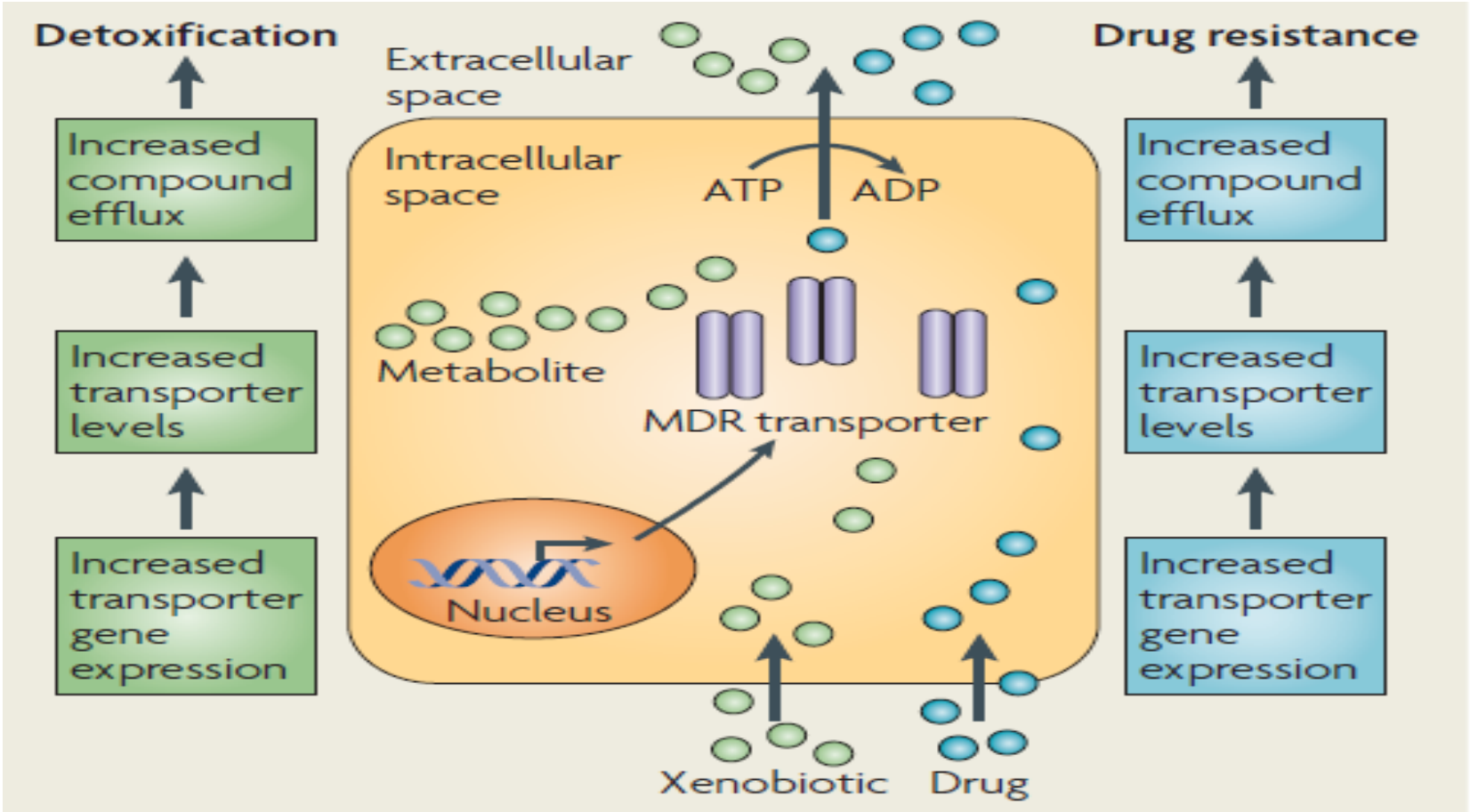


E

The autophagy mechanism of B16 cells induced by anti-bFGF mAb

Drug resistance in cancer chemotherapy



Anti-bFGF mAb reversing multidrug resistance of breast cancer cells

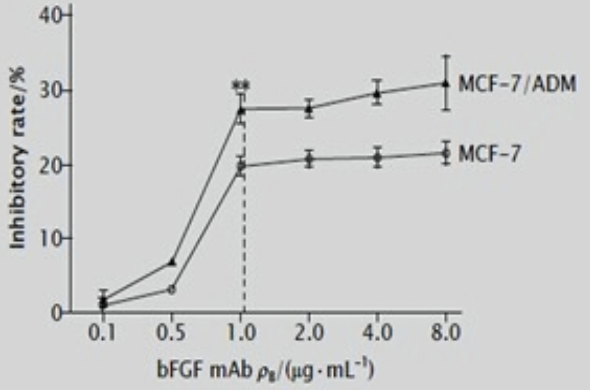


Fig.1 The influence of different concentrations of bFGF mAb (monoclonal antibody to basic fibroblast growth factor) on the growth inhibition rates of MCF-7 cells and MCF-7/ADM (adriamycin) cells were detected by CCK-8 (cell counting kit-8) method (n=3). $^{**}P<0.01$, *vs* MCF-7 cells.

Table 2 The reversal effect of bFGF mAb (monoclonal antibody to basic fibroblast growth factor) on multidrug resistance in breast cancer cell lines was detected by CCK-8 (cell counting kit-8) method.

($\bar{x} \pm s$, n=3)

Group	IC_{50} ρ_B/(μg·mL^{-1})		
	ADM	GEM	OXA
MCF-7	8.35±1.01**	0.27±0.06**	4.46±0.96**
MCF-7/ADM	128.90±4.33	12.06±1.85	38.18±1.73
MCF-7/ADM + 0.5 μg/mL bFGF mAb	99.77±2.79**	9.24±0.78**	33.36±1.20**
MCF-7/ADM + 1.0 μg/mL bFGF mAb	28.88±0.14**	2.84±0.13**	17.49±1.91**
MCF-7/ADM + 0.5 μg/mL mouse IgG	128.22±3.04	12.12±1.36	38.91±1.67
MCF-7/ADM + 1.0 μg/mL mouse IgG	130.86±3.81	12.85±1.41	39.23±1.36

ADM: Adriamycin; GEM: Gemcitabine; OXA: Oxaliplatin; IC_{50}: Half-maximal inhibitory concentration. Mouse IgG was used as a control. $^{**}P<0.01$, *vs* MCF-7/ADM cells.

Table 3 The effects of bFGF mAb (monoclonal antibody to basic fibroblast growth factor) on cell cycle distribution, expression rate of P-gp (permeability-glycoprotein) (B) and accumulation of Rho123 (rhodamine 123) (C) in MCF-7/ADM cells

($\bar{x} \pm s$, n=3)

Group	Cell cycle/%		P-gp expression/%	Fluorescence intensity of Rho123
	G_0/G_1	S		
MCF-7	76.47±1.89**	18.80±1.87**	0.24±0.11**	172.93±13.00**
MCF-7/ADM	44.17±1.96	39.70±1.60	97.90±1.16	8.41±4.22
MCF-7/ADM+1.0 μg/mL bFGF mAb	63.93±1.43**	28.47±2.07**	88.30±1.19**	87.92±16.17**
MCF-7/ADM+1.0 μg/mL mouse IgG	45.25±1.51	37.17±1.87	97.03±1.66	9.37±4.49

ADM: Adriamycin. Mouse IgG was used as a control. $^{**}P<0.01$, *vs* MCF-7/ADM cells.

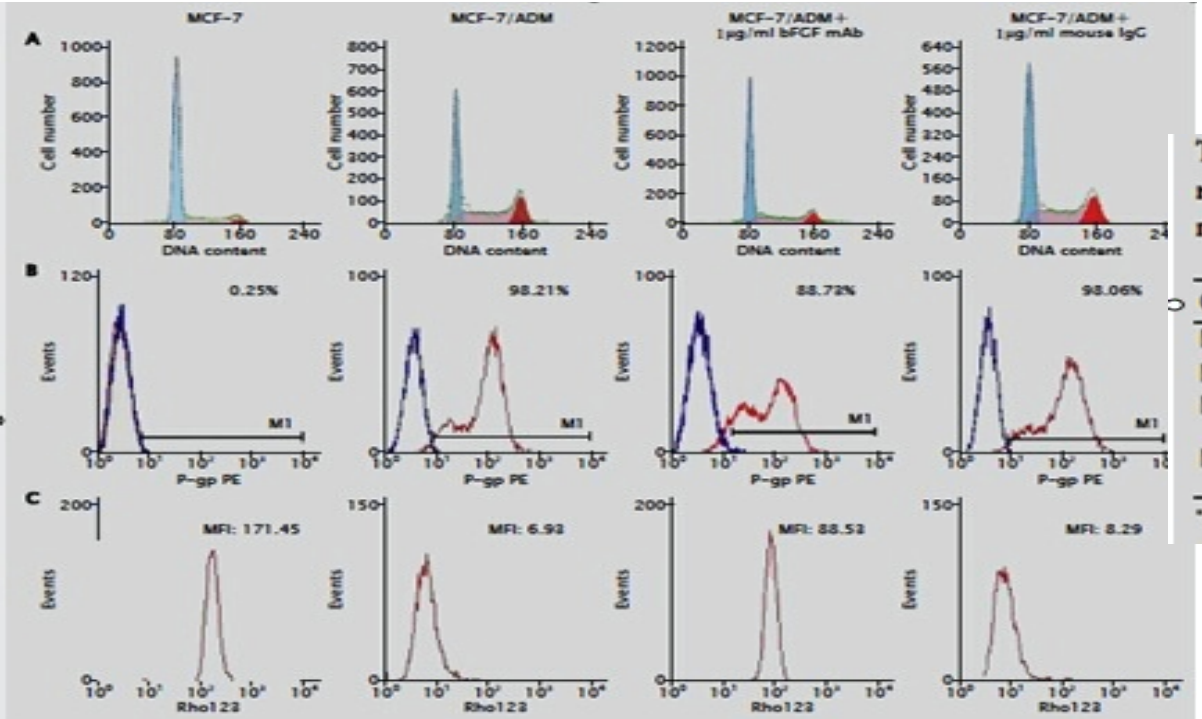


Fig.2 The effects of bFGF mAb (monoclonal antibody to basic fibroblast growth factor) on cell cycle distribution (A), expression rate of P-gp (permeability-glycoprotein) (B) and accumulation of Rho123 (rhodamine 123) (C) in MCF-7/ADM cells were detected by FCM (flow cytometry). ADM: Adriamycin. Mouse IgG was used as a control.

Table 4 The expression levels of MDR 1 (multidrug resistance protein 1) and bFGF (basic fibroblast growth factor) mRNAs in MCF-7 cells and MCF-7/ADM cells ($2^{-\Delta\Delta Ct}$)

($\bar{x} \pm s$, $n=3$)

Group	MDR1 mRNA	bFGF mRNA
MCF-7	1**	1**
MCF-7/ADM	1.66±0.12	36 254.12±48.22
MCF-7/ADM + 1.0 μg/mL bFGF mAb	1.07±0.17**	19 068.13±30.81**
MCF-7/ADM + 1.0 μg/mL mouse IgG	1.53±0.20	36 216.67±42.24

**$P < 0.01$, *vs* MCF-7/ADM cells.

Summary

- Anti-bFGF mAbs display antitumor and antiangiogenic effects *in vitro* and *in vivo*.
- Anti-bFGF mAb is potential therapeutic candidates for melanoma, lung cancer and breast cancer by effectively inhibiting angiogenesis, proliferation, inducing apoptosis and autophagy, reversal of MDR.
- Further preclinical and systematical investigation on anti-bFGF mAb may help to increase efficacy and safety of molecular target treatment.

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