

**FP3-2****“c-met expression and molecular targeting therapy in triple-negative breast cancer”**

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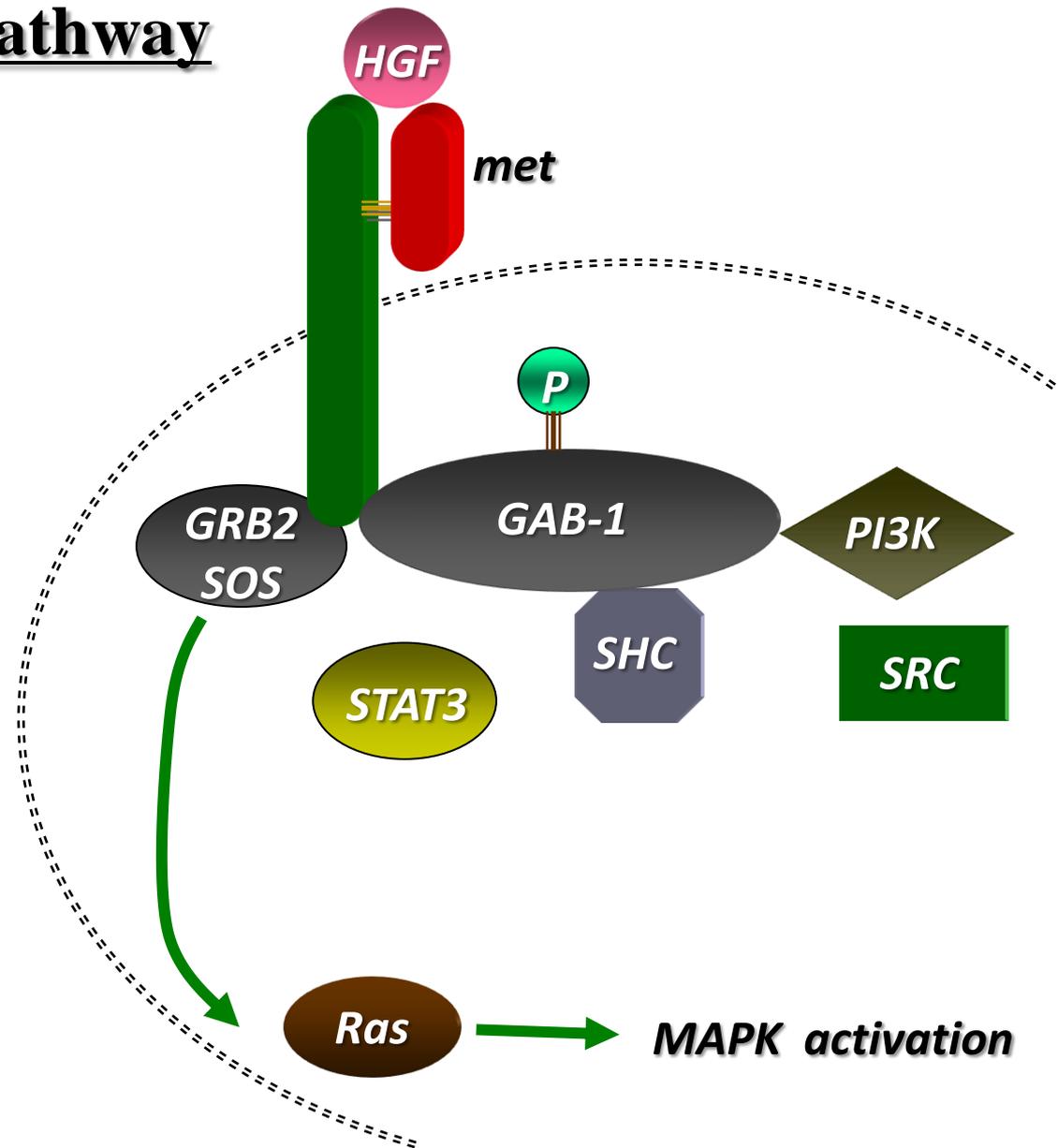
# “Background”

- **The mortality of breast carcinoma is decreasing because of recent developments in diagnostic techniques and therapies; however, the mortality of the triple-negative breast cancer (TNBC) remains poor. TNBC a poorly characterized subtype of tumor with no validated clinical assay to identify them.**
- **Reportedly, the prognosis of breast cancer is correlated with HGF/c-met coexpression and c-met expression. c-met signaling plays an important role in the proliferation of breast cancer cells. However, little is known about the c-met expression levels of TNBC.**
- **Here, we examined the correlation between TNBC and c-met expression and the effects of c-met inhibitors in TNBC cell lines.**



## c-met signal pathway

**HGF, which specifically binds to the c-met receptor, is involved in the proliferation, migration, and morphological transformation of cancer cells.**





# “Materials and Methods”

## ➤ Breast Cancer Cell Lines

**TNBC  
cell lines**

- MDA-MB 231; HR(-), HER2(-)
- OCUB-2; HR(-), HER2(-)

**non-TNBC  
cell lines**

- MCF-7; HR(+), HER2(-)
- OCUB-1; HR(-), HER2(+)

## ➤ c-met inhibitors

- SU11274
- PHA665752

**c-met inhibitors on the proliferation of breast cancer cell lines were examined.**



# “Materials and Methods”

➤ A total of 1,036 patients who had undergone resection of a primary breast cancer at our institute were enrolled.

**TNBC**                      **190 cases (18.3%)**  
**non-TNBC**                **846 cases (81.7%)**

➤ **Immunohistochemistry**

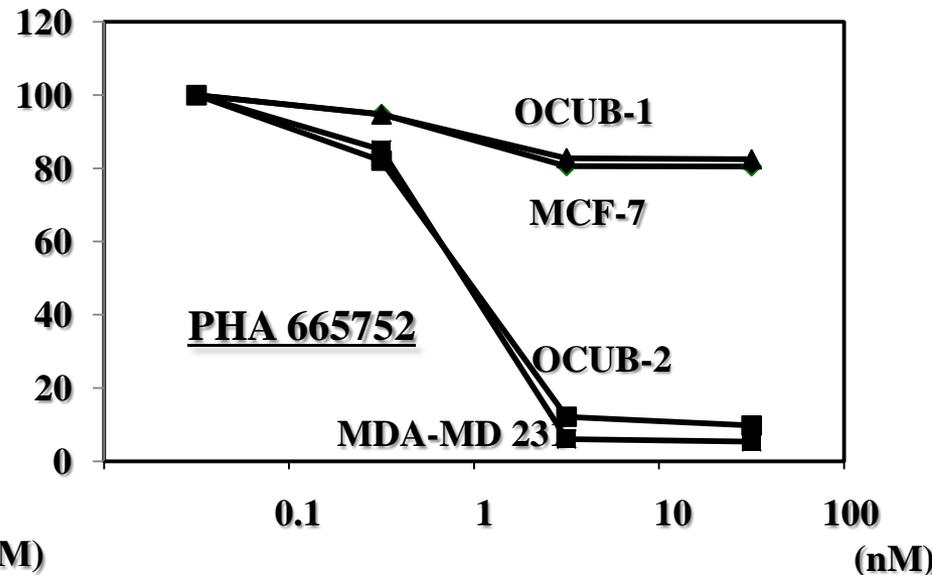
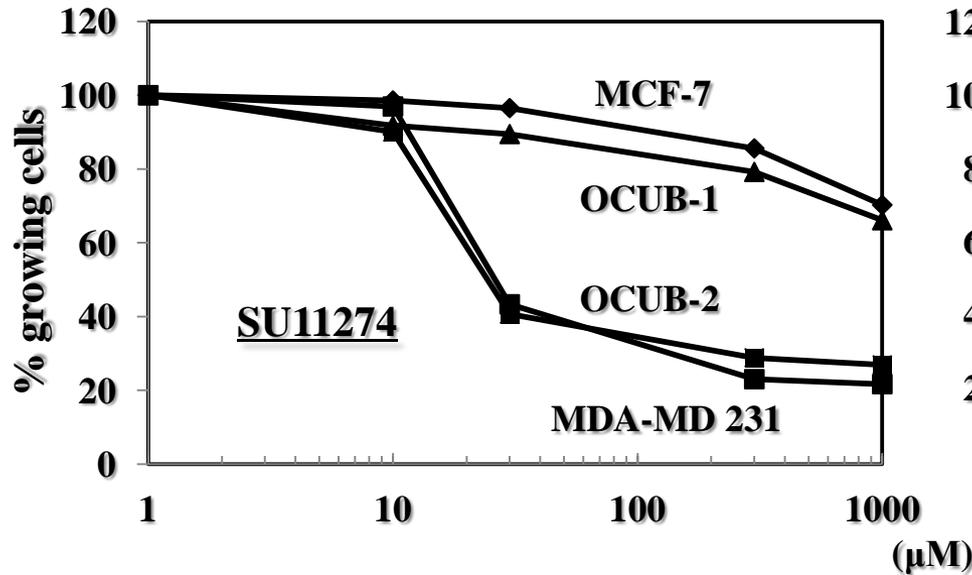
antibody	clone	dilution	source	staining pattern	cutoff values
ER	clone 1D5	1:80	Dako Cytomation	Nucleus	0%
PR	clone PgR 636	1:100	Dako Cytomation	Nucleus	0%
HER2	-	1:300	Dako Cytomation	Membrane	10%
c-met	sc-162	1:100	SANTA CRUZ	Cytoplasm	30%

**ER / PR / HER2 status and c-met expression were assessed by immunohistochemistry.**



# c-met kinase inhibitors inhibited the proliferation of TNBC cell lines.

*(Proliferation assay)*

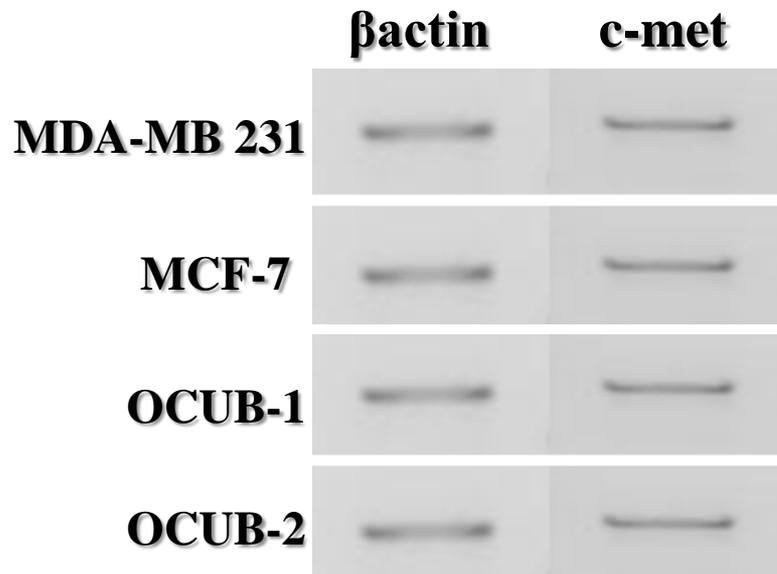




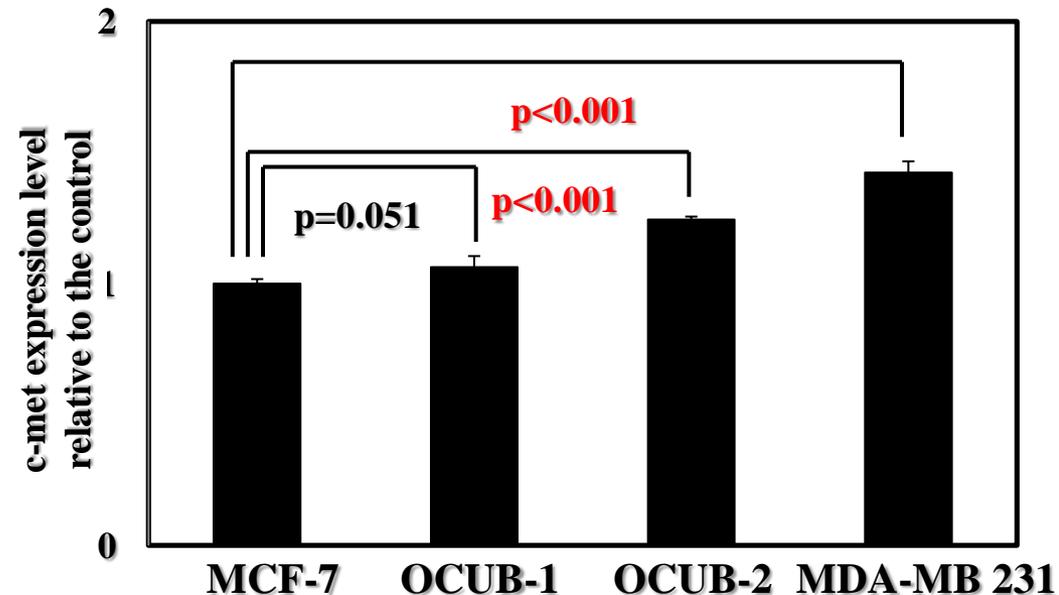
# Breast cancer cell lines expressed c-met mRNA.

## c-met Expression

(RT-PCR)



(qRT-PCR)

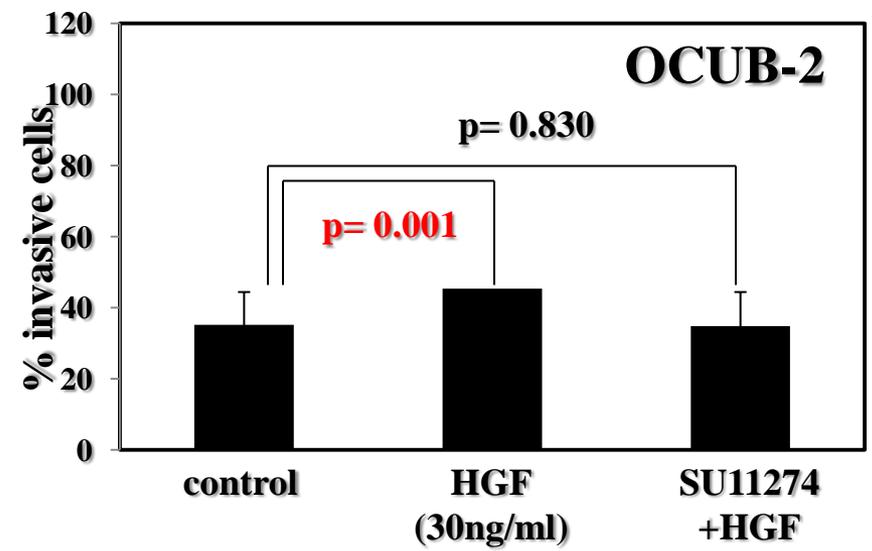
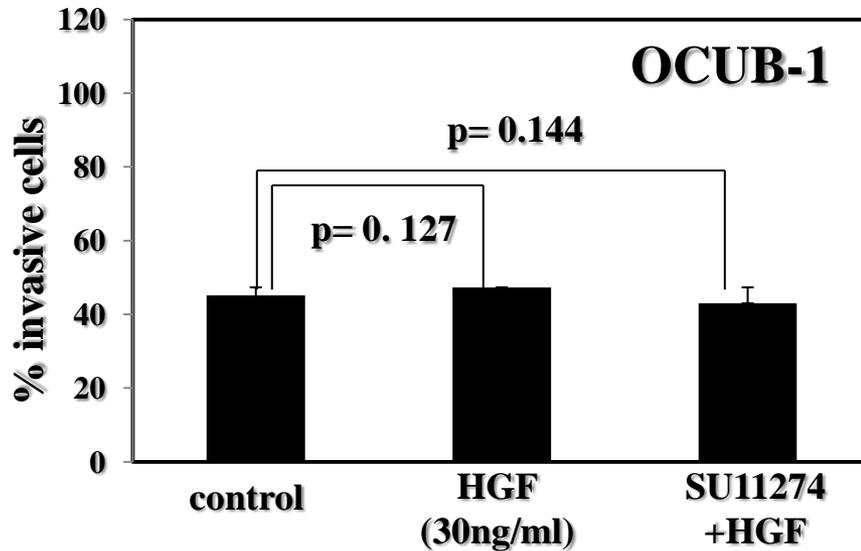
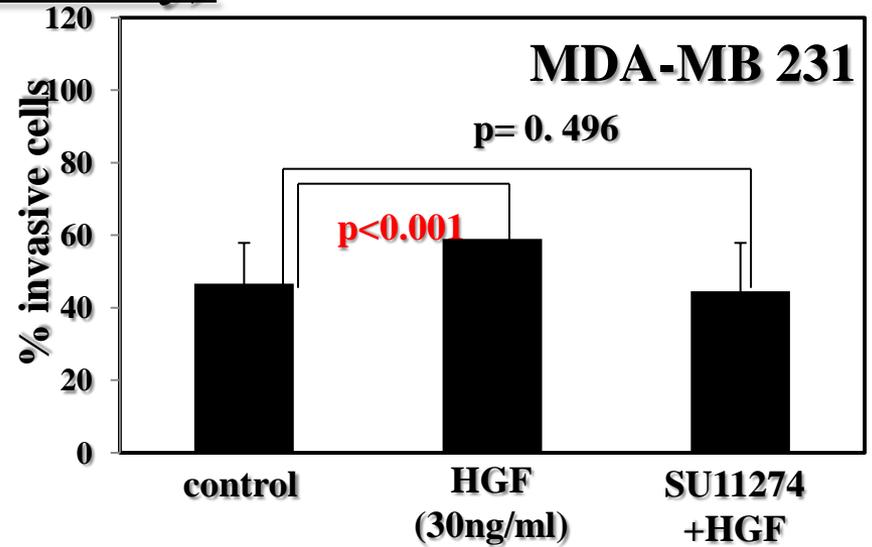
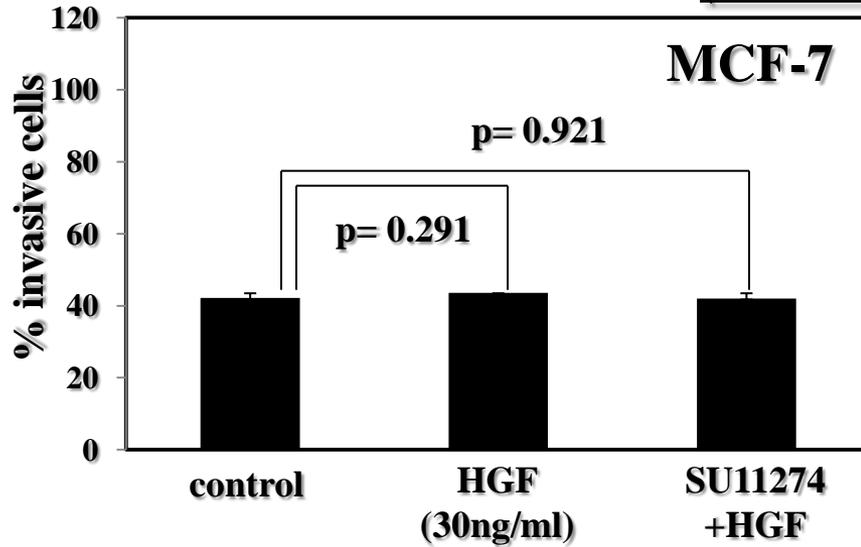


The quantitative RT-PCR showed that the expression level of c-met mRNA was significantly high in MDA-MB 231 and OCUB-2 cells, compared with the expression in OCUB-1 and MCF-7.



**c-met kinase inhibitors SU11274 inhibited the invasion of TNBC cell lines.**

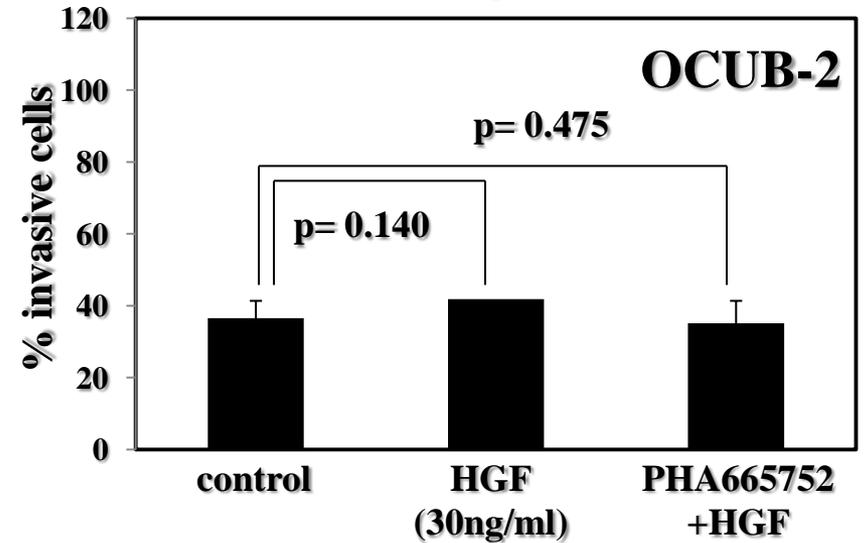
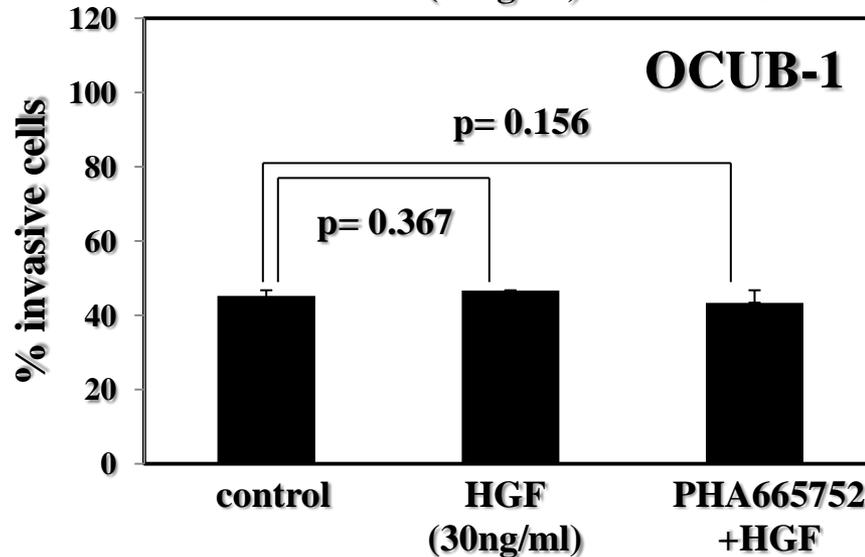
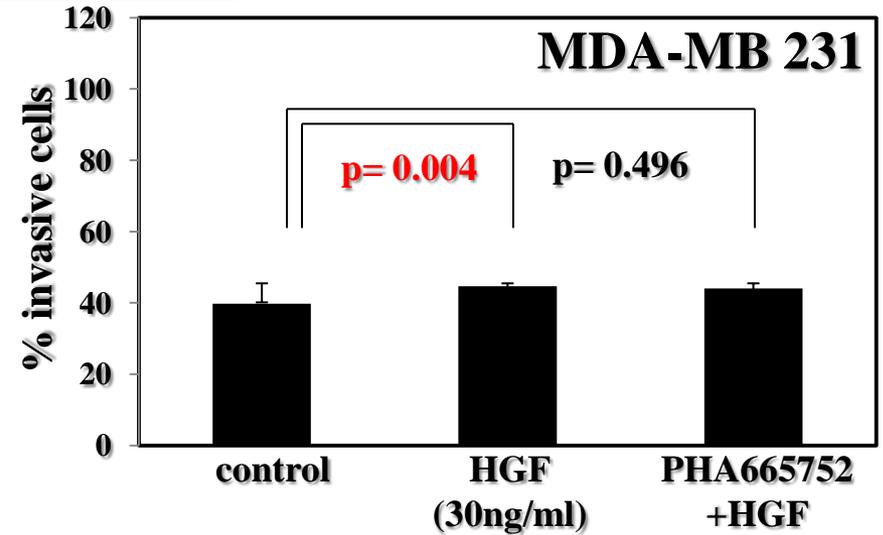
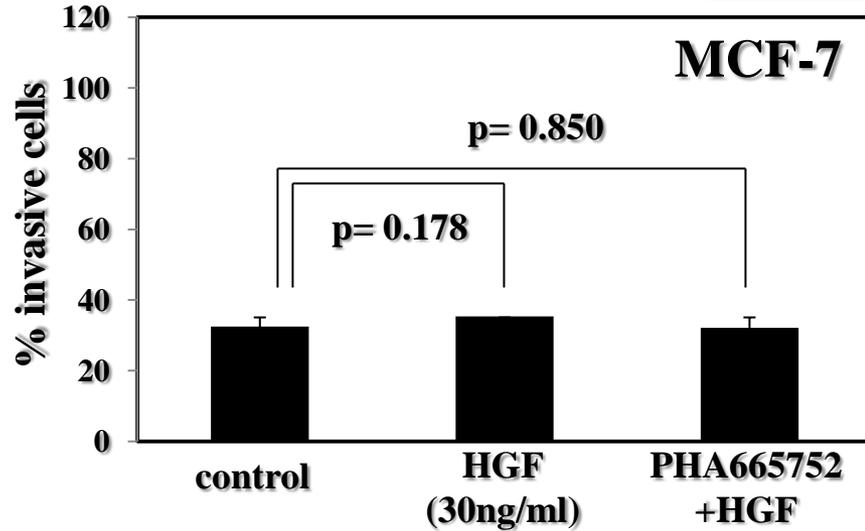
*(Invasion assay)*





# c-met kinase inhibitors PHA665752 inhibited the invasion of TNBC cell lines.

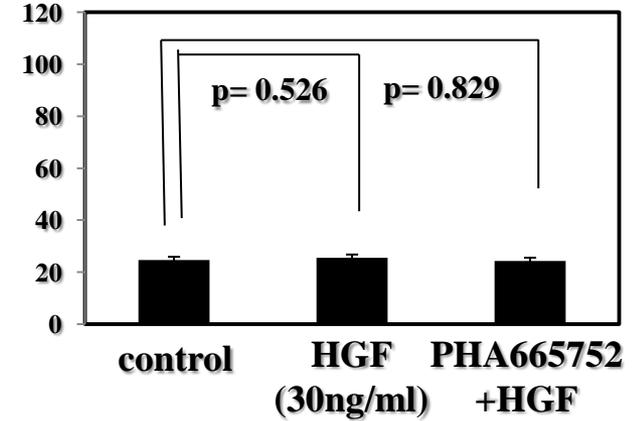
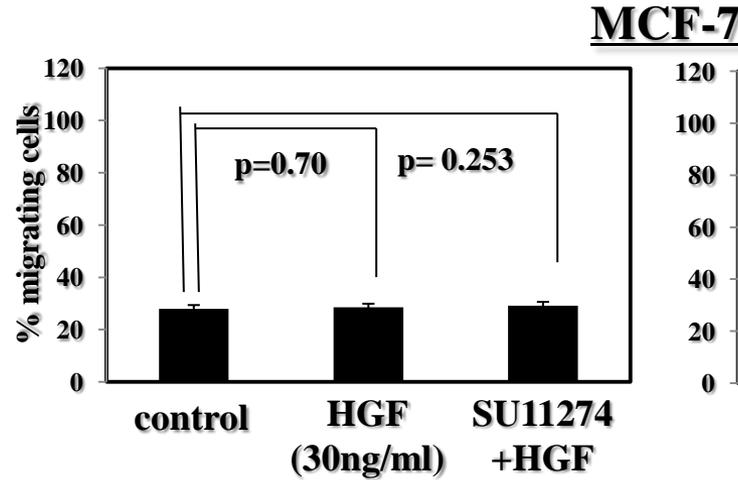
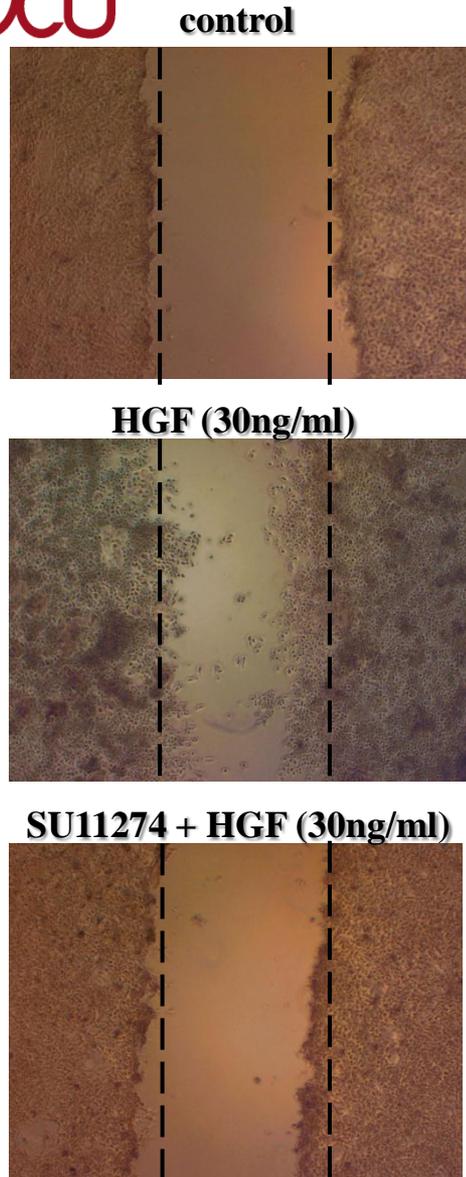
## *(Invasion assay)*



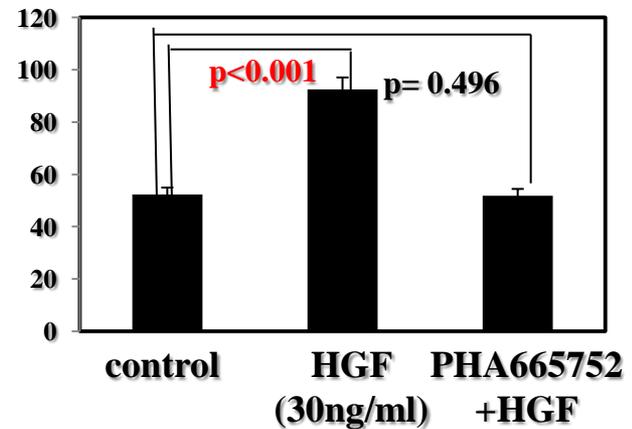
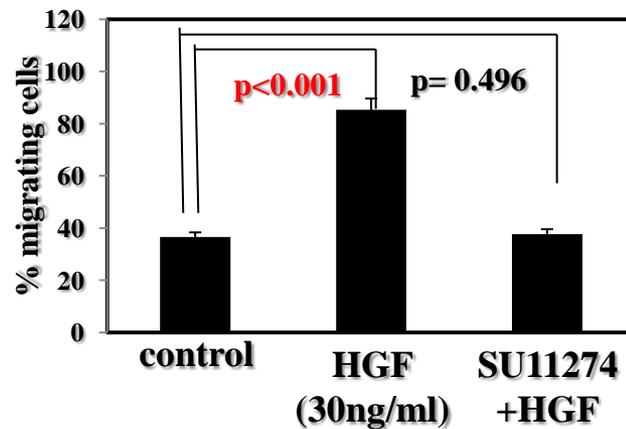


**c-met kinase inhibitors inhibited the migration of TNBC cell lines.**  
*(Wound healing assay)*

**MDA-MB 231**



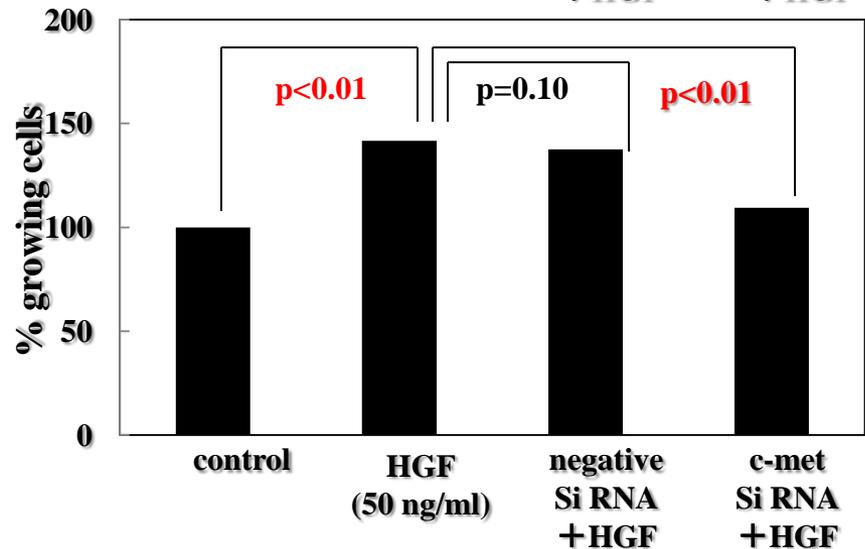
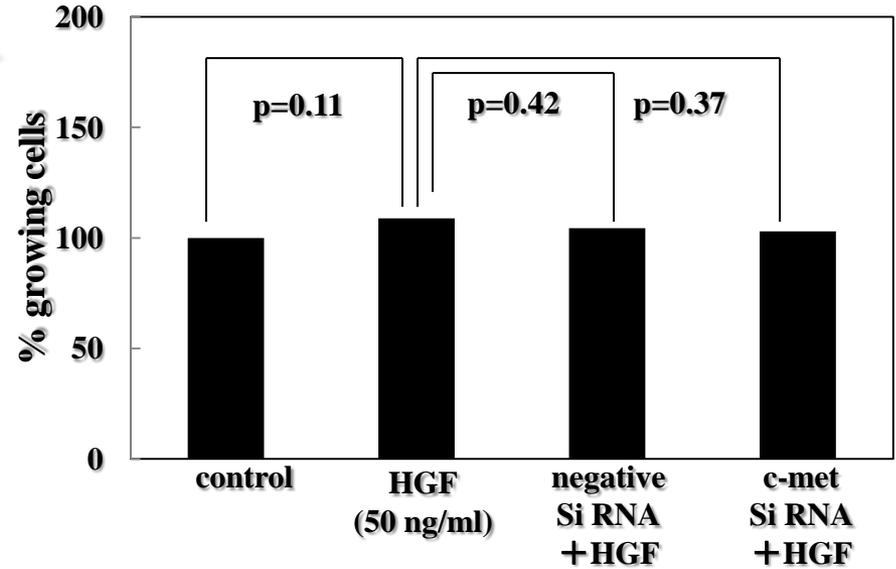
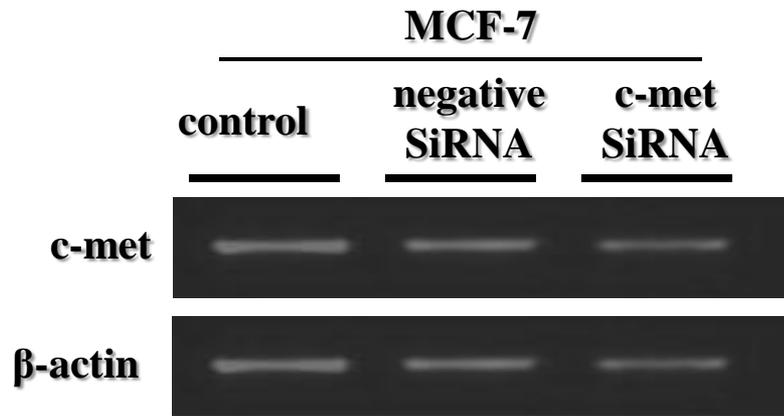
**MDA-MB 231**





# c-met SiRNA inhibited the proliferation of TNBC cell lines.

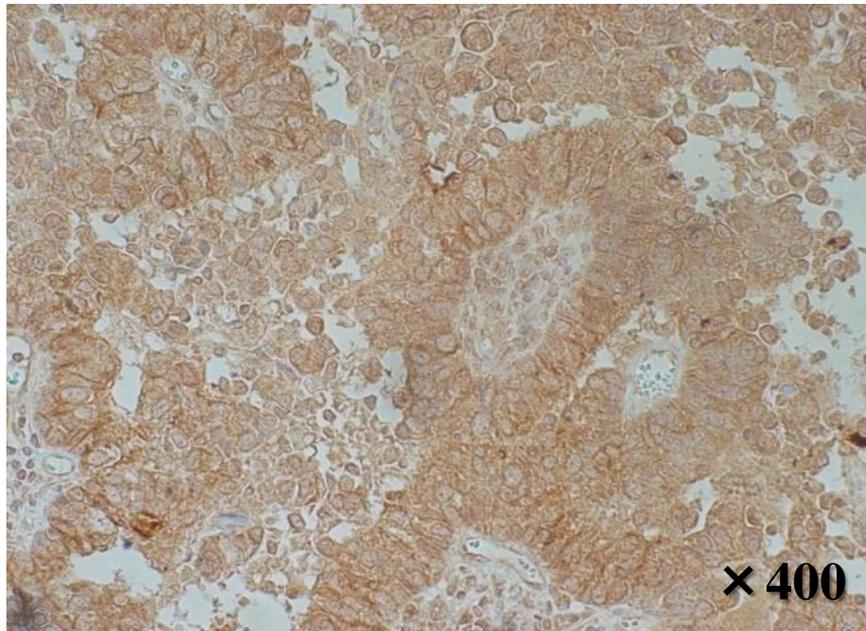
## Effect of c-met siRNA on expression mRNA



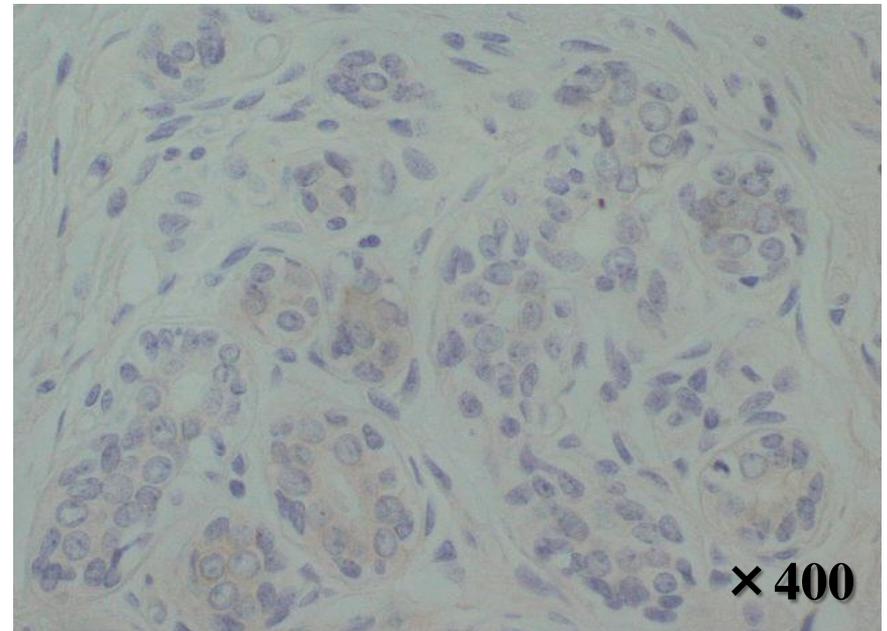


## c-met expression (*Immunohistochemistry*)

**positive**



**negative**

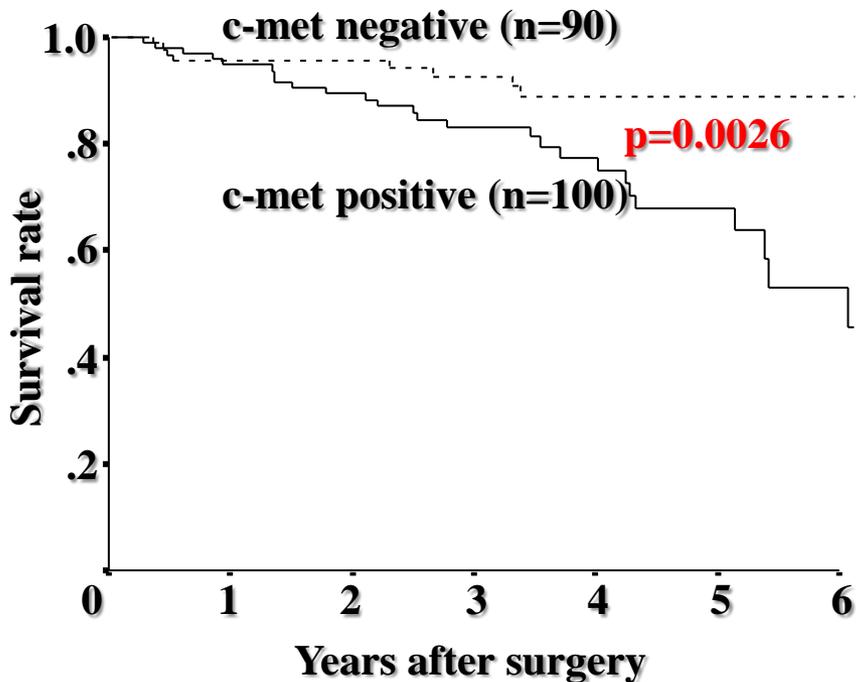




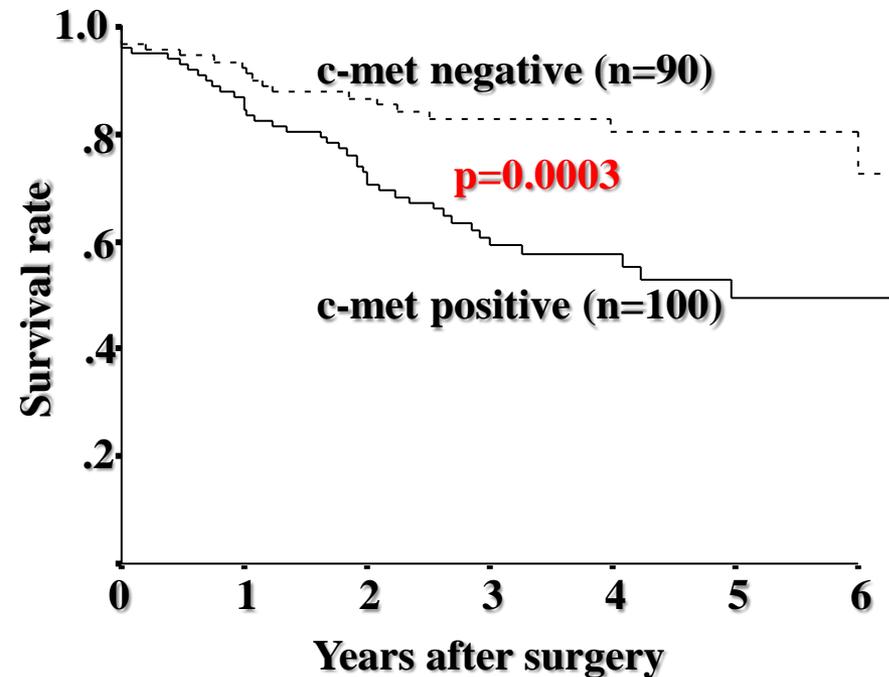
## Correlation between c-met positive group and overall survival and disease-free interval.

### Triple negative breast cancers (n=190)

#### Overall Survival



#### Disease Free Interval



**The prognosis of patients with c-met positive in 190 TNBC expression was significantly worse than that of those with c-met negative.**



# Multivariate analysis with respect to overall survival in 190 TNBC.

	Univariate analysis			Multivariate analysis		
	Odds ratio	95% CI	p value	Odds ratio	95% CI	p value
<b>Stage</b>						
1 vs 2, 3, 4	<b>2.54</b>	<b>1.04-6.22</b>	<b>0.041</b>	<b>0.37</b>	<b>0.06-2.39</b>	<b>0.298</b>
<b>Tumor size</b>						
≤2 cm vs >2 cm	<b>2.46</b>	<b>1.11-5.45</b>	<b>0.027</b>	<b>2.59</b>	<b>0.60-11.22</b>	<b>0.202</b>
<b>Lymph node status</b>						
N0 vs N1, N2, N3	<b>3.39</b>	<b>1.67-6.88</b>	<b>0.001</b>	<b>3.17</b>	<b>1.26-7.89</b>	<b>0.014</b>
<b>Lymphvascular invasion</b>						
Negative vs Positive	<b>1.84</b>	<b>0.94-3.58</b>	<b>0.074</b>	<b>1.31</b>	<b>0.65-2.66</b>	<b>0.448</b>
<b>c-met</b>						
Negative vs Positive	<b>3.17</b>	<b>1.44-7.01</b>	<b>0.004</b>	<b>2.51</b>	<b>1.12-5.65</b>	<b>0.026</b>

**In TNBC, cases with c-met expression was an independent indicator of a poor prognosis by multivariate analyses.**



## **“Summary”**

- c-met was expressed in the TNBC cell lines, whose proliferation was enhanced by HGF. c-met kinase inhibitors and c-met SiRNA inhibited the proliferation of TNBC cell lines.**
- Clinical samples were immunohistologically examined to demonstrate that c-met expression is an independent poor prognostic factor in TNBC.**

## **“Conclusion”**

**c-met expression is a potential molecular target and useful in classifying TNBC.**