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Effect of scaffold and growth factors on anti-cancer drug screening with multicellular spheroids: mimicking in vivo response!

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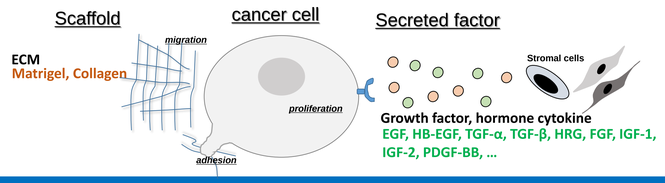
Introduction

Cancer microenvironment is being increasingly recognized as a key factor. In the past, several 3D cell culture models have been tested as the powerful method for reproducing cancer microenvironment. However, in some cases, the growth and drug sensitivity of cells grown on anchorage-independent 3D culture models have been different to that of cells grown *in vivo*. Further, development of the 3D culture model including the physical (the cell-ECM interaction (scaffold)) and secreted factor (growth factor, cytokine) are essential for mimicking cancer microenvironment *in vitro*. Therefore, we explored the effect of the growth factor dependent proliferation and drug sensitivity in scaffold/scaffold-free culture models, and try to understand which method is mimicking *in vivo* response due to stimulation and suitable for anti-cancer drug development.

Key player of cancer microenvironment



Various tumor models

Model type		*In vivo*	*In vitro*, 3D Scaffold		*In vitro*, 3D Scaffold-free	*In vitro*, 2D
		Xenografts	Matrigel	NanoCulture Plate(NCP)	Low adhesion round plate	Tissue culture plate
		-	ECM-embedded growth factor contained	a gel free minimal attachment	a gel free floating	a gel free conventional plate
Inherent Features	Reproducibility	+	-	+	-	+++
Tumor Biomimetics	Drug penetration	+++	+++	++	++	---
	ECM similarity	++	++	+	+	---
	Phenotype similarity (proliferation, drug sensitivity)	++	?	?	?	?

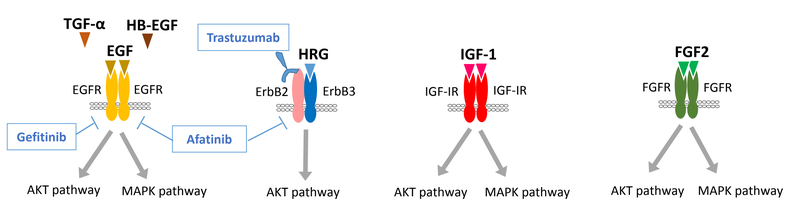
Figure 1. Summary of tumor models and these main characteristics

Strategy

Subjects: Which factors are important for mimicking *in vivo*?
Which methods are better for anti-cancer drugs development?

1) **Evaluate growth factor response on cell proliferation in various *in vitro* cell culture models, scaffold/scaffold-free 3D model and 2D model.**
2) **Compare the drug sensitivity in several culture conditions.**
3) **Compare our *in vitro* results with *in vivo* references.**

Growth factors and inhibitors/anti-caner drugs

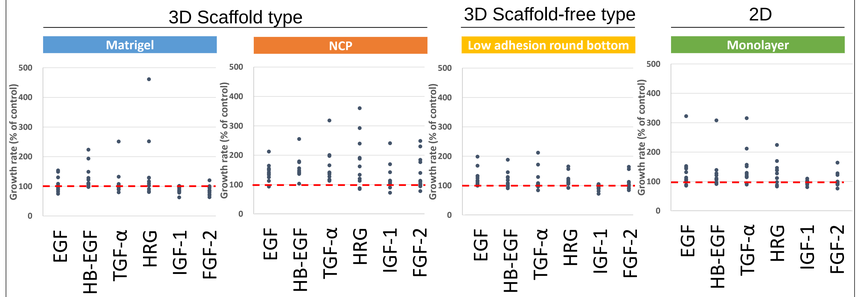


Cell lines on NCP actively proliferated according to various growth factor stimuli

Table 1. Growth factor response on cell proliferation under *in vitro* culture models.
Eleven cancer cell lines were cultured with various growth factors (10 nM) under different *in vitro* cell culture conditions, as indicated. Growth rate (% of control) was calculated as growth factor responsiveness on cell proliferation.

		3D Scaffold type												3D Scaffold-free type						2D					
		Matrigel						NCP						Low adhesion round bottom						Monolayer					
Cell line	Tissue	EGF	HB-EGF	TGF-a	HRG	IGF-1	FGF-2	EGF	HB-EGF	TGF-a	HRG	IGF-1	FGF-2	EGF	HB-EGF	TGF-a	HRG	IGF-1	FGF-2	EGF	HB-EGF	TGF-a	HRG	IGF-1	FGF-2
BT474	Mammary Gland	154	194	251	91	63	94	213	255	319	293	241	230	118	109	105	113	94	103	153	94	157	170	81	90
BT549	Mammary Gland	96	107	103	81	83	73	165	144	198	120	108	94	133	146	172	110	103	110	148	111	212	88	93	90
JIMT-1	Mammary Gland	102	109	101	102	92	96	152	176	166	191	142	184	199	188	212	157	100	164	113	121	119	112	103	97
MCF-7	Mammary Gland	103	110	100	129	102	101	133	138	136	240	114	177	109	96	97	93	90	90	106	110	116	139	95	122
MDA-MB-231	Mammary Gland	91	103	91	110	99	85	93	177	117	85	90	98	105	119	107	107	105	113	86	91	89	98	100	92
MDA-MB-435s	Mammary Gland	82	111	89	129	88	63	113	103	116	113	105	103	100	102	103	106	100	98	108	107	115	111	110	100
SKBR-3	Mammary Gland	130	122	98	117	79	84	137	136	127	162	111	113	115	98	84	124	72	91	144	139	151	146	98	97
T-47D	Mammary Gland	109	128	97	252	86	120	155	156	142	360	113	249	168	129	109	165	90	156	132	127	125	224	96	164
ZR7530	Mammary Gland	88	224	108	461	97	79	125	142	140	188	169	140	126	91	104	93	82	84	89	97	130	127	89	76
OVCAR-3	Ovary	75	98	80	84	81	87	150	149	201	134	102	109	105	107	108	119	95	104	322	308	316	138	100	128
SKOV-3	Ovary	149	149	132	103	95	70	142	179	113	88	72	78	133	127	129	110	105	101	102	106	94	83	97	94

High / Low



HB-EGF stimulated SKOV-3 showed higher sensitivity to gefitinib on Matrigel and NCP culture conditions

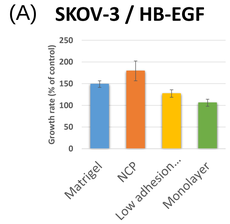
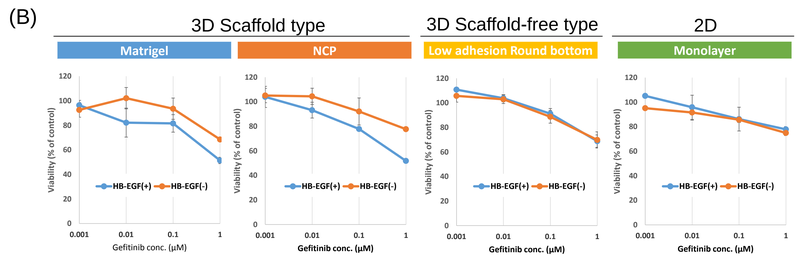


Figure 3. Effect of Gefitinib on HB-EGF treated SKOV-3 cultured in different 3D models and monolayer condition.

(A) Growth enhancement by HB-EGF was observed in Matrigel and NCP culture conditions.
(B) HB-EGF stimulation changed SKOV-3 cellular character to be more sensitive to Gefitinib in Matrigel and NCP culture conditions.



HRG stimulated MCF-7 showed higher sensitivity to afatinib and trastuzumab on NCP

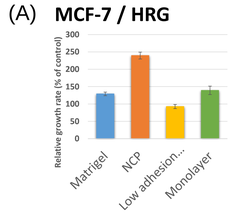
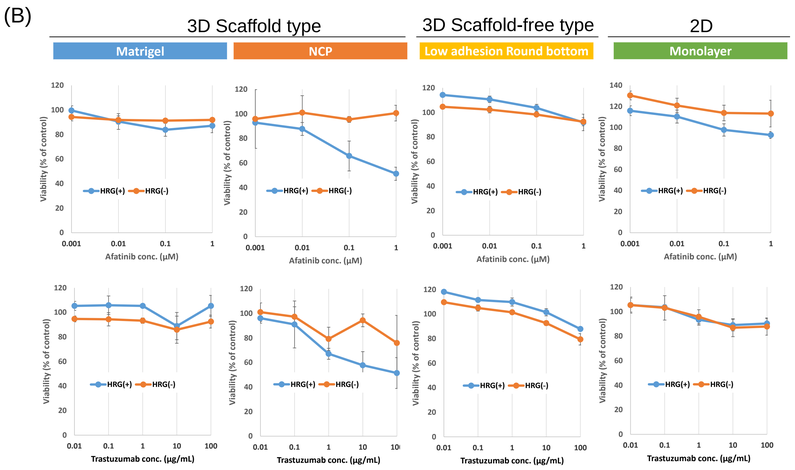


Figure 4. Effect of afatinib and trastuzumab on HRG treated MCF-7 cultured in different 3D models and monolayer condition.

(A) Growth enhancement by HRG was observed better in NCP culture condition.
(B) HRG stimulation changed MCF-7 cellular character to be more sensitive to afatinib and trastuzumab only in NCP culture condition.



Discussion

Comparison of *in vitro* results and xenograft model:
In xenograft model, growth factor responsiveness on cell proliferation and drug sensitivity were indicated in
Reference 1: J Cell Sci. 2009 Dec 1;122(Pt 23):4277-86.
- HB-EGF overexpressing-SKOV-3 injected into nude mice grew faster than parental SKOV-3.
Reference 2: J Clin Oncol. 2007 Jul 1;25(19):2656-63.
- HRG overexpressing MCF-7 developed tumor faster in mice than wild-type MCF-7.
- Trastuzumab inhibited tumor growth in mice.

These results may show that **cancer cell proliferation and drug sensitivity on NCP is similar to that on xenograft tumor model**.

Table 2. *in vivo* similarity in tumor models

	Xenografts	Matrigel	NanoCulture Plate(NCP)	Low adhesion round plate	Tissue culture plate
Phenotype similarity (proliferation, drug sensitivity)	++	+	++	-	-

Conclusion

- Growth factor stimulation on NCP 3D model promoted cell proliferation and increased drug sensitivity.
- Growth factor responsiveness on cell proliferation and drug sensitivity on NCP 3D model were similar to *in vivo* behavior.
- These results demonstrated that scaffold and growth factor facilitate mimicking the cancer microenvironment *in vitro*.
- Therefore, it is obvious that NCP can be used as a suitable 3D culture model for mimicking *in vivo* response and anti-cancer drug development.