Therapeutic Target for Cancer Metastasis : Regulation of TGF-β by Post Translational Modification

M2 Fujiwara 2018.11.07 (Wed)

Contents

Cancer metastasis

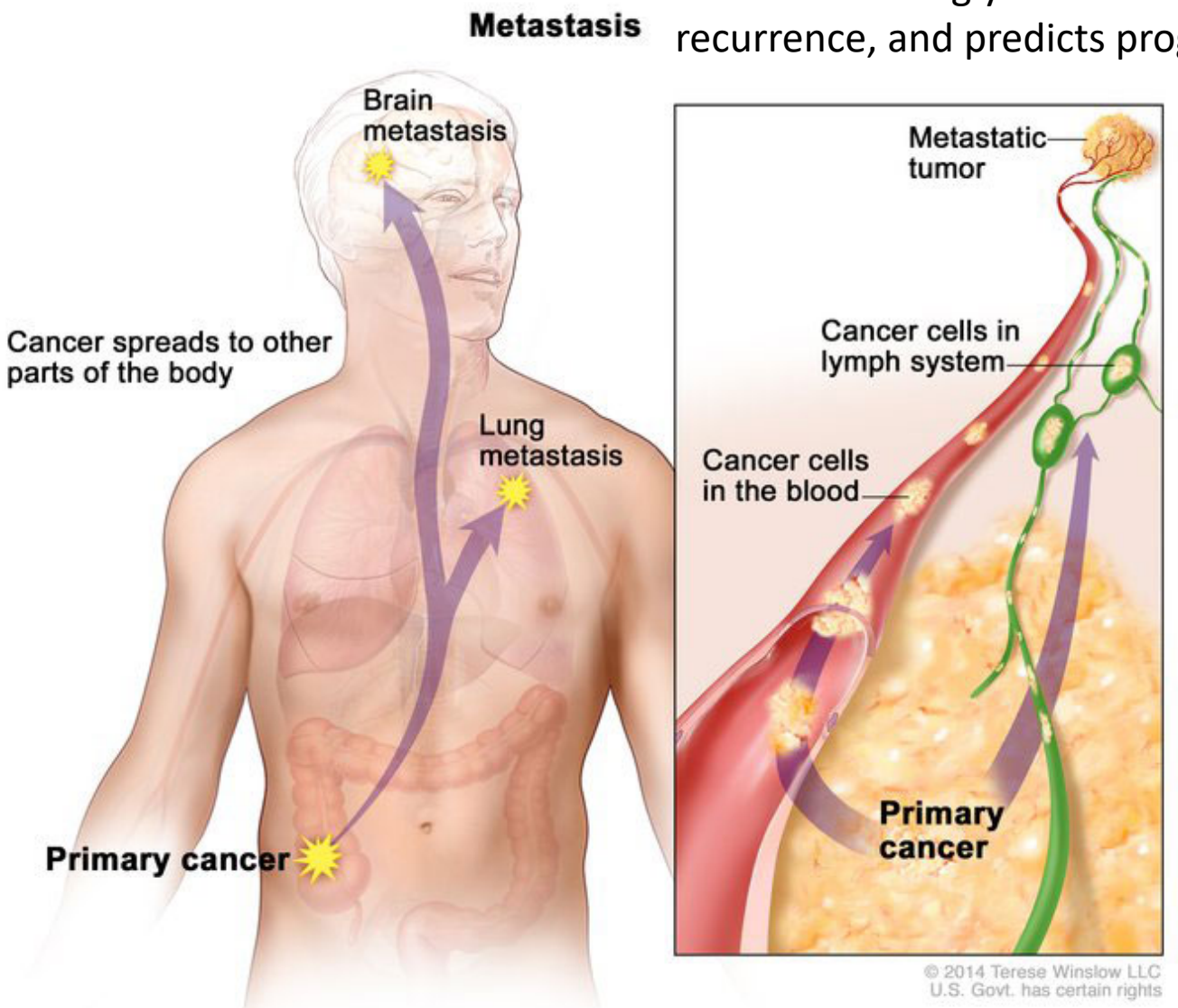
- Cancer metastasis
- Epithelial-to-Mesenchymal Transfer

Metastasis inhibition – Blocking TGFβ pathway

- TGFβ (inducing EMT) is promising target for cancer metastasis
- Control of TGFβ signaling by Ubiquitination

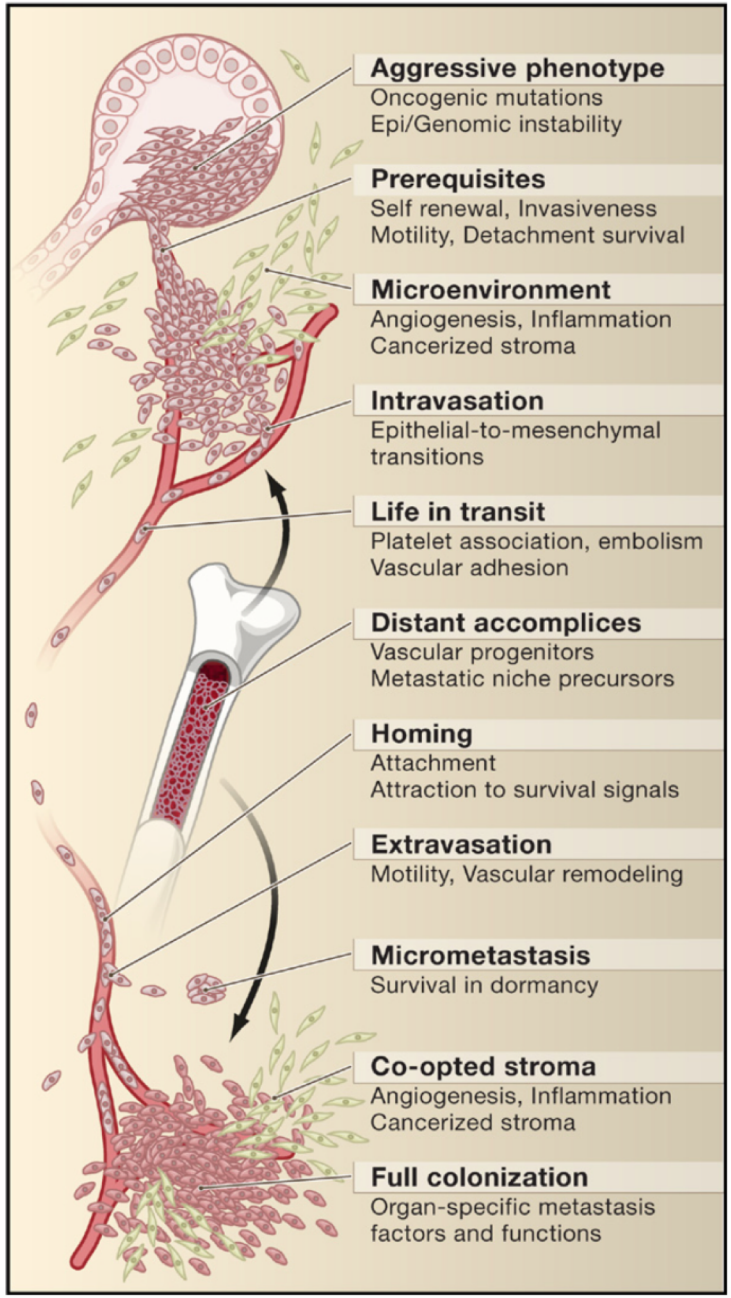
Cancer metastasis

- Cause of 90% of deaths from solid tumors
- Gene profiling for metastasis in primary tumors correlates strongly with the likelihood of metastatic recurrence, and predicts prognosis of the patients.



https://www.cancer.gov/types/metastatic-cancer

Prerequisites for Metastasis



Cancer cells must evade or co-opt multiple rules and barriers that were refined for long years of organismal evolution.

- Tolerance for apoptosis Inappropriate proliferation...
  Tolerance for tumor microenvironment (Hypoxia, reactive oxygen, limited nutrients)

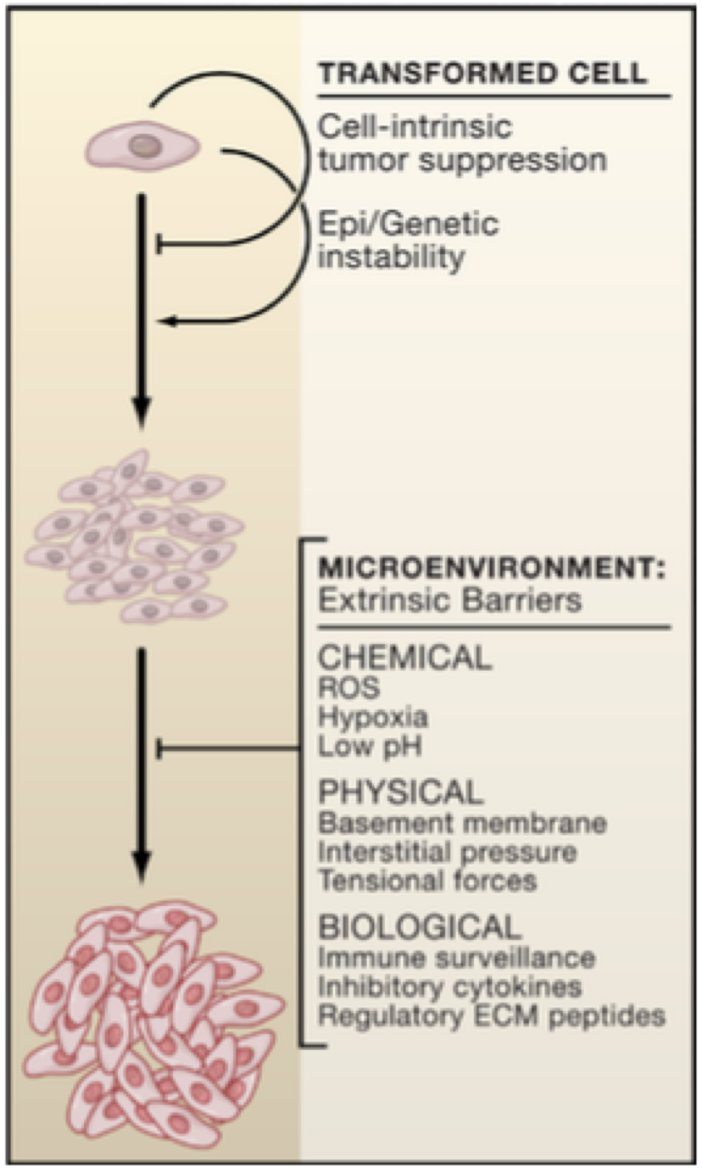
- Loss of cellular adhesion (E-cadherin), Increased motility and invasiveness
  Collusion with Immune response (NF-κB, MMP9, HGF), stromal cells (TGF-β, IL-10, IL-23...)

- Entry and survival in the circulation (lymph, vessel)
  Angiogenesis, co-opting blood platelets.

- Exit into new tissue and colonization
  Integrins for secondary organs, Cancer stem cells

G. P. Gupta et al. Cell *127,* **679** (2006)

Cancer Evolution for Metastasis



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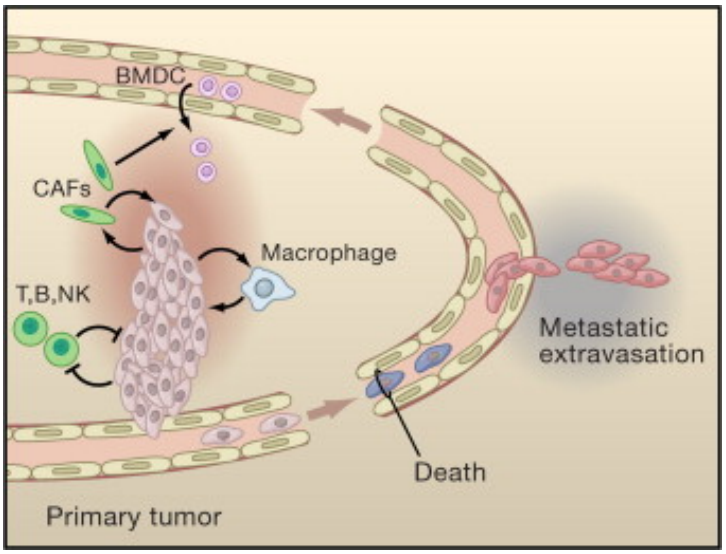

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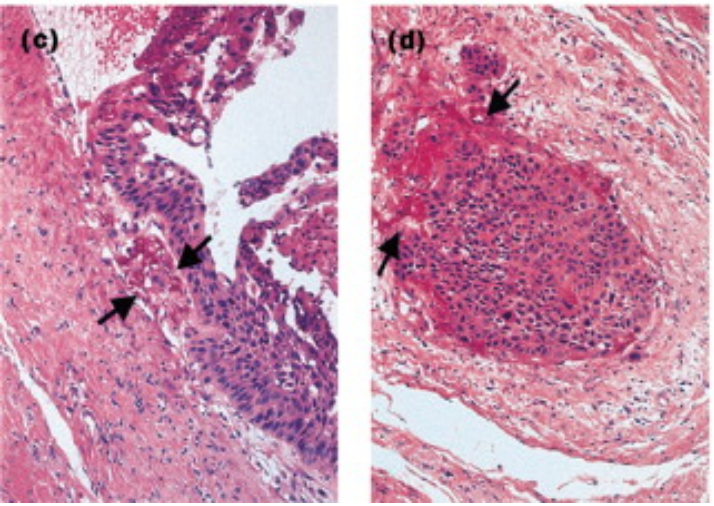

Carcinoma located within blood vessels. Squamous-cell carcinoma (扁平上皮癌) of tongue (舌). Fibrin deposition (arrows) and tissue-like architecture.

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D. J. Ruiter, et al. THE LANCET Oncology 2, 2001, 109

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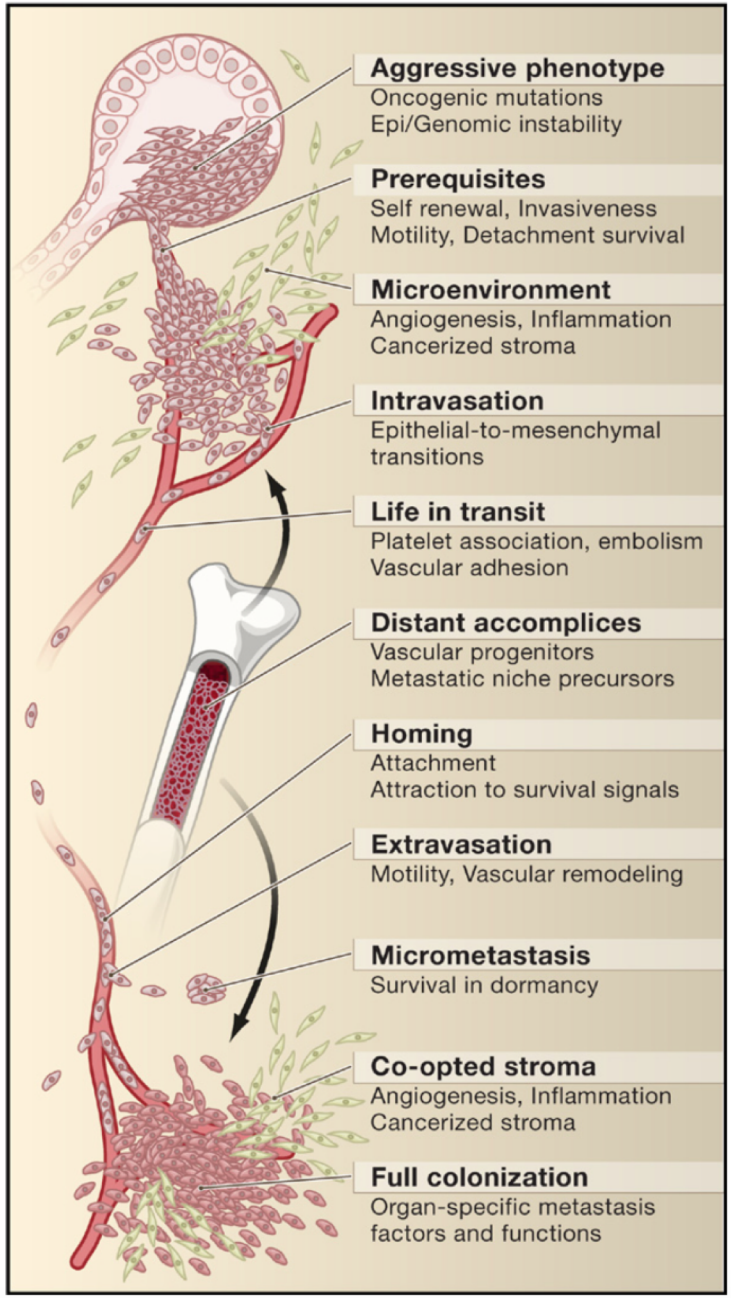

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Metastasis inhibition – Blocking TGFβ pathway

- TGFβ (inducing EMT) is promising target for cancer metastasis
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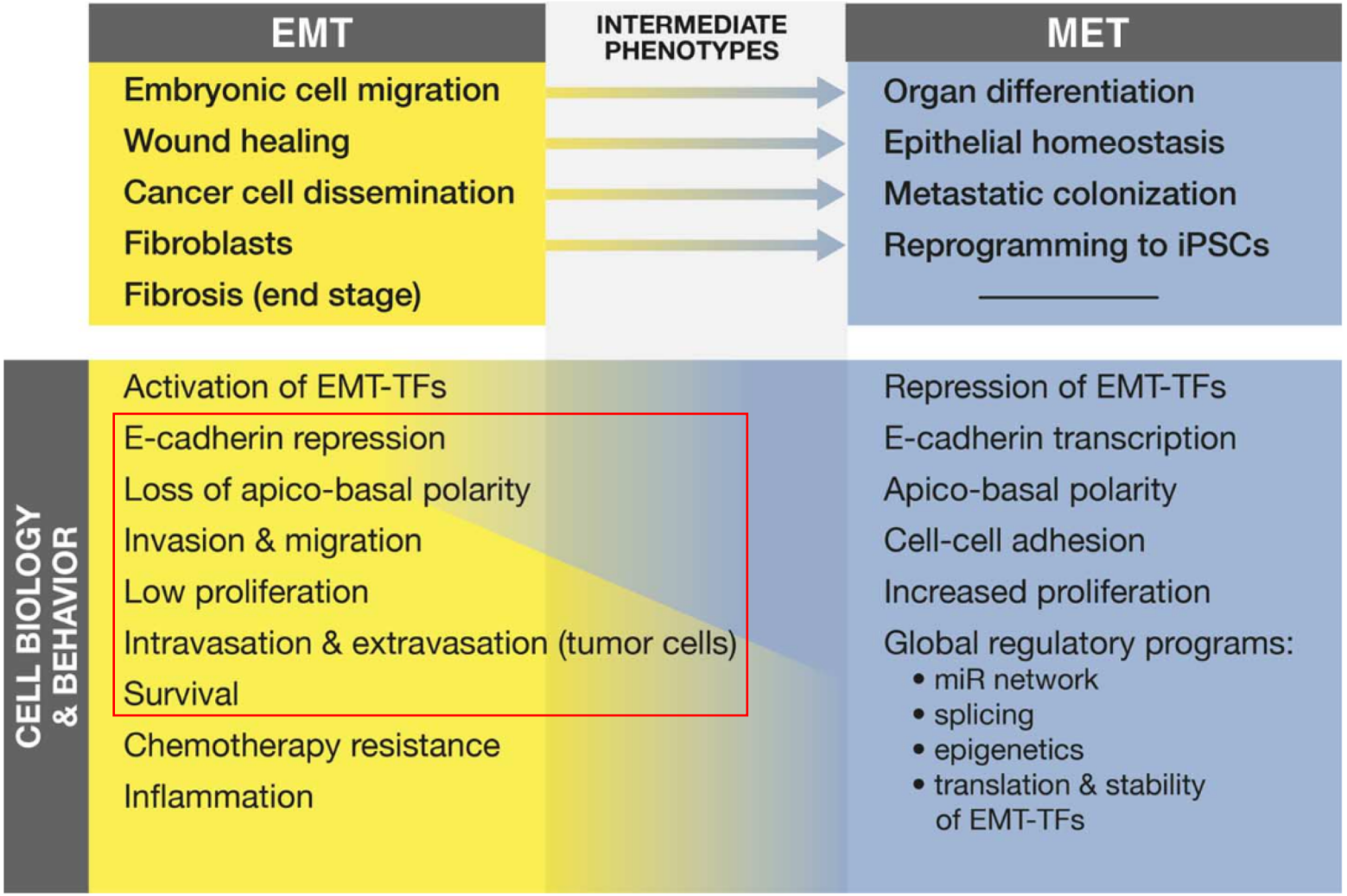
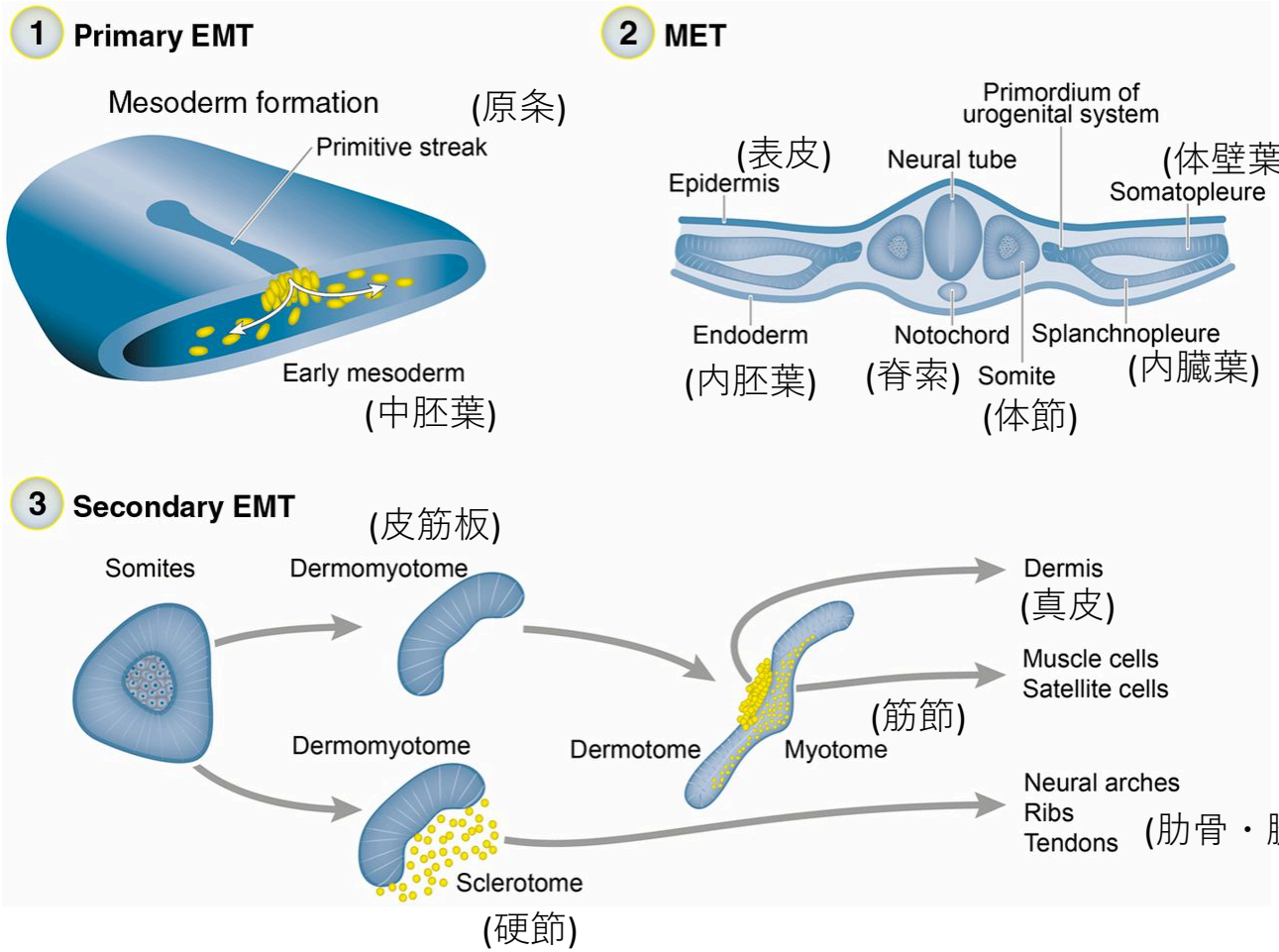
Cancer cells escape from carcinomas have characteristics of “EMT”



- (EMT-induced cells gain property of stem cells)

M Angela Nieto, *Science* 342, 1234850 (2013)

Epithelial-to-Mesenchymal Transition (EMT)



EMT
- Embryonic cell migration
- Wound healing
- Cancer cell dissemination
- Fibroblast formation

MET
- Organ differentiation
- Epithelial homeostasis
- Metastatic colonization

Features of EMT
- N-cadherin, vimentin... (mesenchymal marker)
- EMT-TFs (transcription factor), such as ZEB1, Twist, Snail...

Features of MET
- E-cadherin, α-catenin... (Epithelial marker)

*mesoderm -- > muscle, body cavity(体腔)...
*endoderm -- > digestive tract, liver, ...

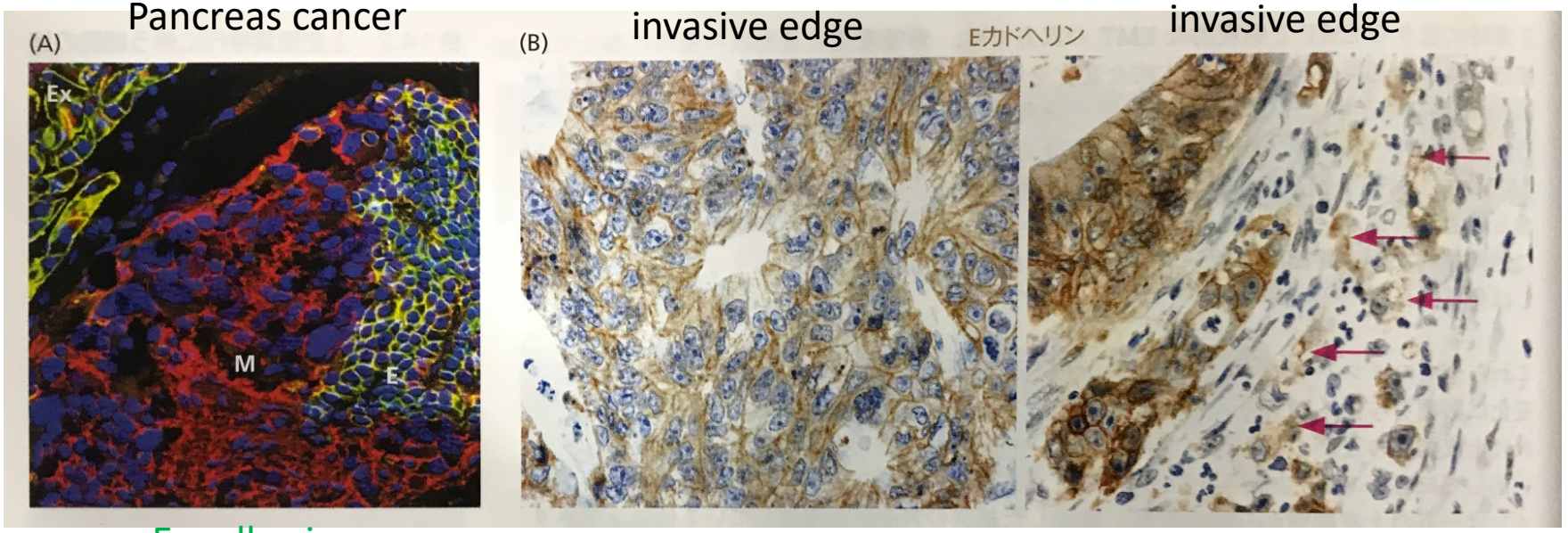
Embryonic development undergo 2 or 3 rounds of EMT and MET to form each organs.

EMT may occur in cancer cells...

Pancreas cancer

Intestinal cancer
invasive edge

Intestinal cancer
invasive edge



E-cadherin
β-catenin

Decrease of Epithelial marker is found in tumor cells.
And the many features of EMT match the requirement of cancer metastasis, so it is thought that EMT should occur in cancer metastasis.

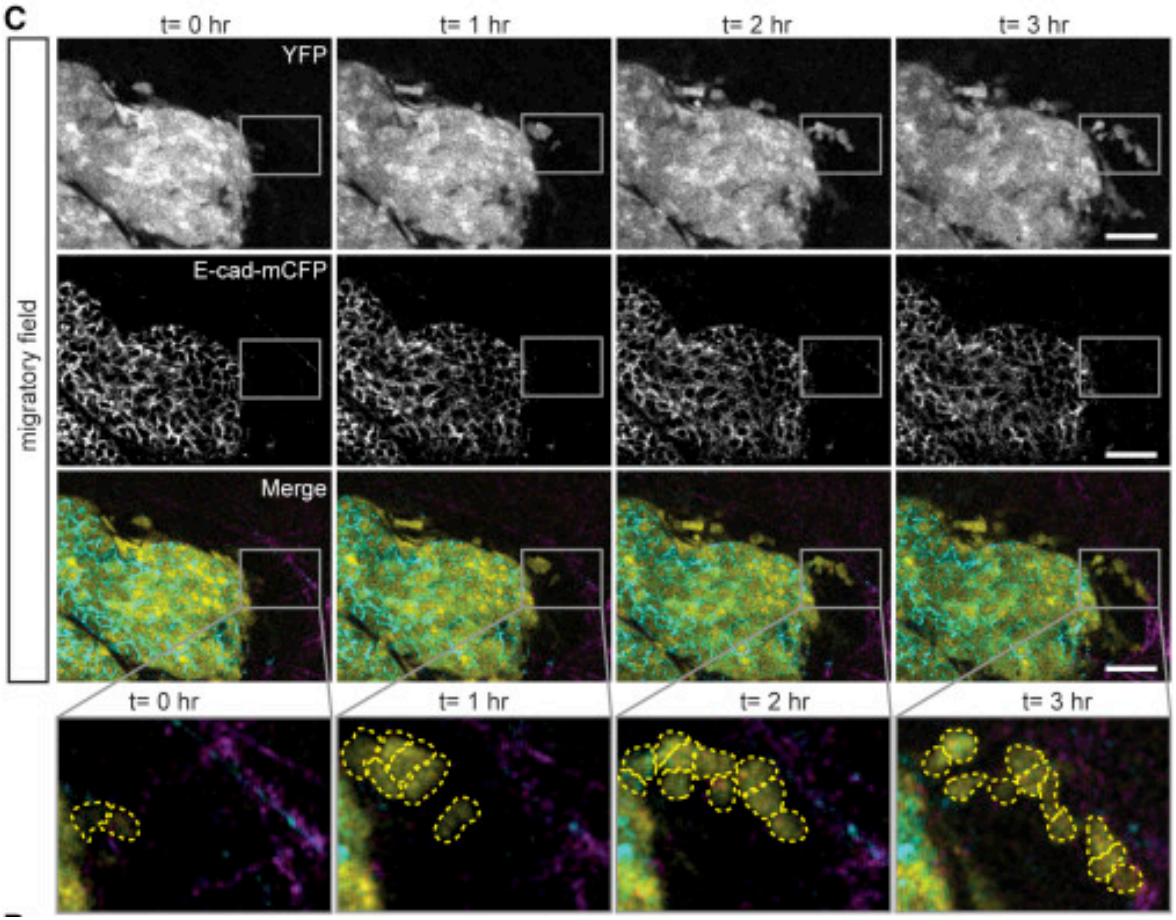
Features of EMT
・N-cadherin, vimentin... (mesenchymal marker)
・EMT-TFs (transcription factor), such as ZEB1, Twist, Snail...
Features of MET
・E-cadherin, α-catenin... (Epithelial marker)

In model mice, EMT occur in metastasis.

E-cad^{HI} tumor cells expressing E-cad-mCFP & YFP

non-obese diabetic SCID IL-2 receptor gammma chain knockout mice

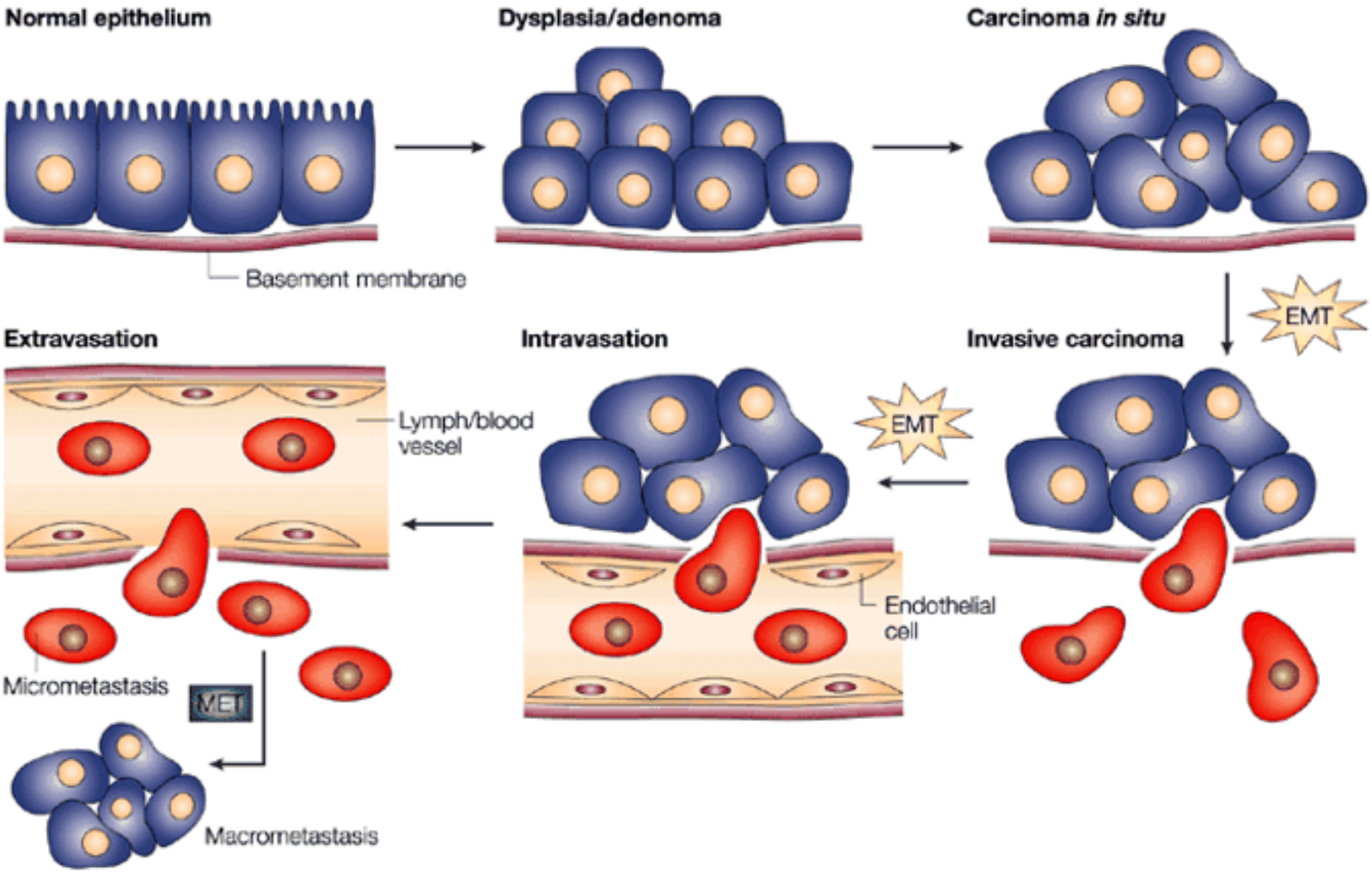
E-cad^{HI} ... Epithelial expression
E-cad^{LO} ... Mesenchymal expression
Yellow ... tumor cells



Motile tumor cells have undergone EMT without artificially modifying EMT regulators.

E. Beerling et al. Cell Reports 14, 2281 (2016)

Assumed role of EMT and MET in cancer metastasis



J. P. Thiery Nature Reviews Cancer 2, 442 (2002)

Short summary

・Cancer metastasis is critical problem for patients, because it causes death or poor prognosis.

・In metastasis step, tumor cells should clear many hurdles from various environmental pressures.

・Cancer cells evolve through their own epi/genomc instability or interaction with environment, and then gain mesenchymal features to survive harsh conditions.

・EMT program, which is primary known in embryonic cells, is now thought to be the important step in tumor metastasis.

Contents

EMT is new target for cancer?

- EMT is essential for progression and metastasis in the tumor metastasis.
- EMT-TFs are associated with chemo-resistance in cancer (see table below).

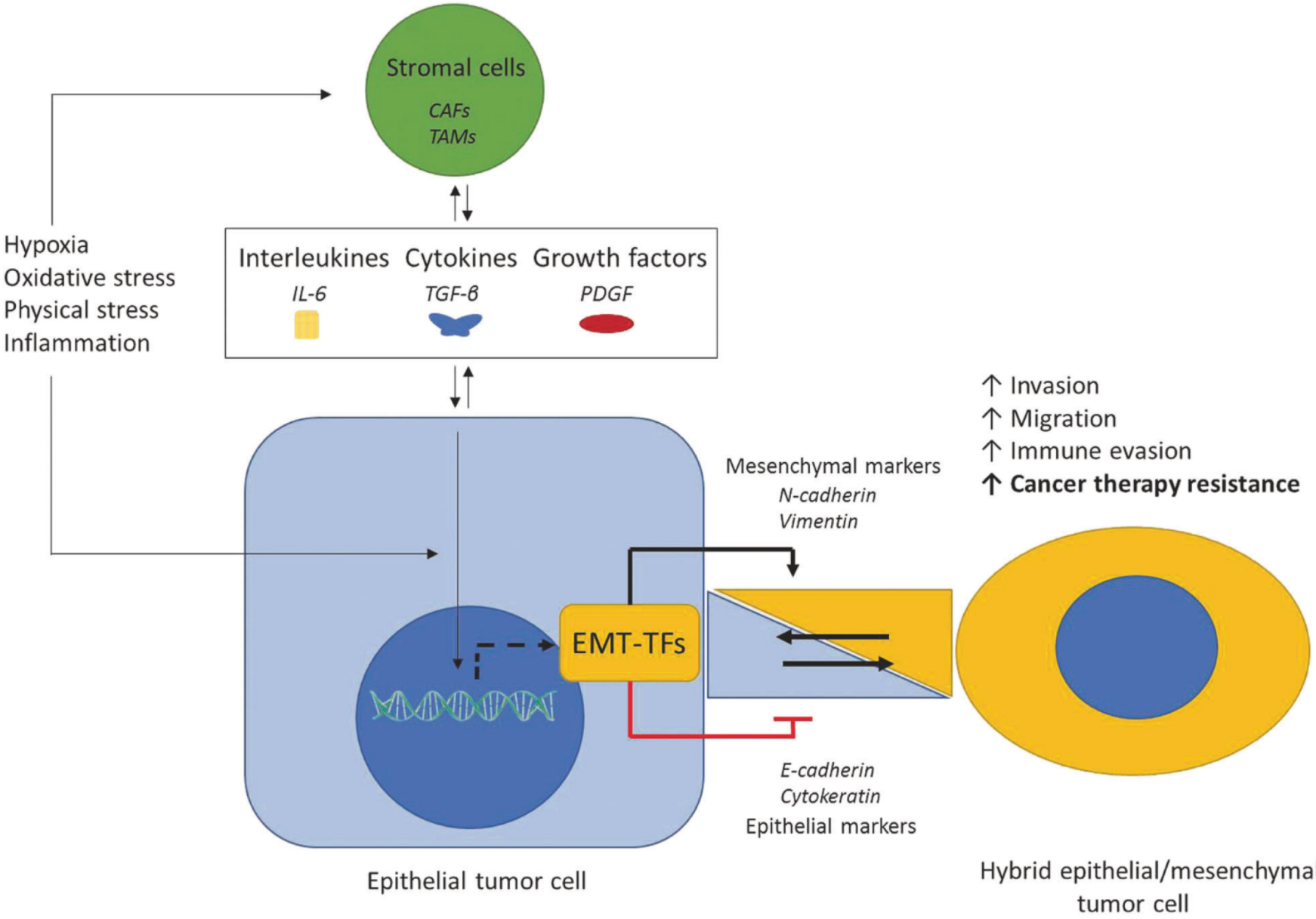
EMT-TF	Drug [83–129]	Cell line
TWIST1	Paclitaxel	Head and neck squamous cell carcinoma
	Paclitaxel, vincristine, cisplatin	Nasopharyngeal carcinoma
	5-Fluorouracil	Oral squamous cell carcinoma
	Paclitaxel, doxorubicin, letrozole, tamoxifen, fulvestrant, trastuzumab	Breast carcinoma
	5-Fluorouracil, gemcitabine	Hepatocellular carcinoma
	Cisplatin	Pancreatic cancer
	Anthracycline	Bladder cancer
	Paclitaxel	Epithelial ovarian carcinoma
	5-Fluorouracil	Colon cancer
	Oxaliplatin	Colorectal cancer
TWIST2	Cisplatin	Epithelial ovarian cancer
SNAI1	Cisplatin, gefitinib, erlotinib	Head and neck squamous cell carcinoma
	Cisplatin	Nasopharyngeal carcinoma
	Cisplatin	Non-small cell lung cancer
	Doxorubicin, camptothecin, 5-Fluorouracil	Breast carcinoma
	Oxaliplatin	Hepatocellular carcinoma
	5-Fluorouracil, gemcitabine	Pancreatic cancer
	Cisplatin	Prostatic cancer
	Radiation, paclitaxel, cisplatin	Epithelial ovarian cancer
	Oxaliplatin	Colorectal cancer
SNAI2	Cisplatin	Nasopharyngeal carcinoma
	Gefitinib	Lung adenocarcinoma
	Tamoxifen, fulvestrant, trastuzumab	Breast carcinoma
	Gemcitabine	Pancreatic cancer
	Radiation, paclitaxel, cisplatin	Epithelial ovarian cancer
ZEB1	Cisplatin	Nasopharyngeal carcinoma
	Nintedanib, erlotinib, crizotinib, gefitinib	Non-small cell lung cancer
	Docetaxel	Lung adenocarcinoma
	Radiation, doxorubicin, tamoxifen, fulvestrant, trastuzumab	Breast carcinoma

EMT-TFs can affect...

- drug metabolism by changing the expression of drug-transporters
- render tumor cells less sensitive to therapy induced apoptosis
- kinase inhibitor resistance (?)

EMT can result in extensive changes in the cytoskeleton and the tumor cell surface repertoire, which alters tumor immunogenicity on the cell surfaces.

Molecular mechanism of EMT



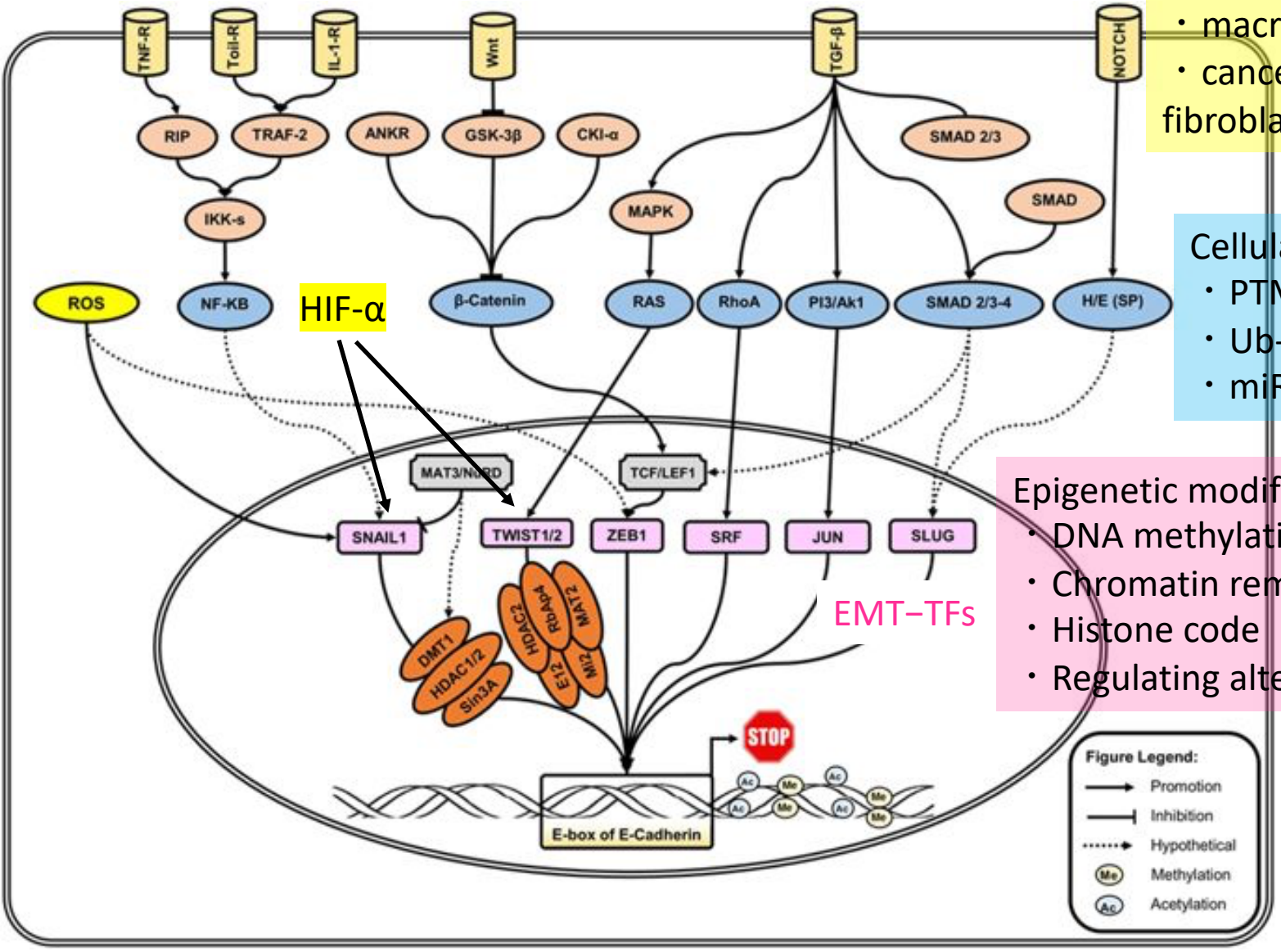
J. Staalduinen et al. Oncogene (2018)

EMT-inducible signal pathway

Cellular crosstalk from
- macrophages,
- cancer-associated fibroblasts

Cellular environment
- PTM
- Ub-degradation
- miRNA reguation

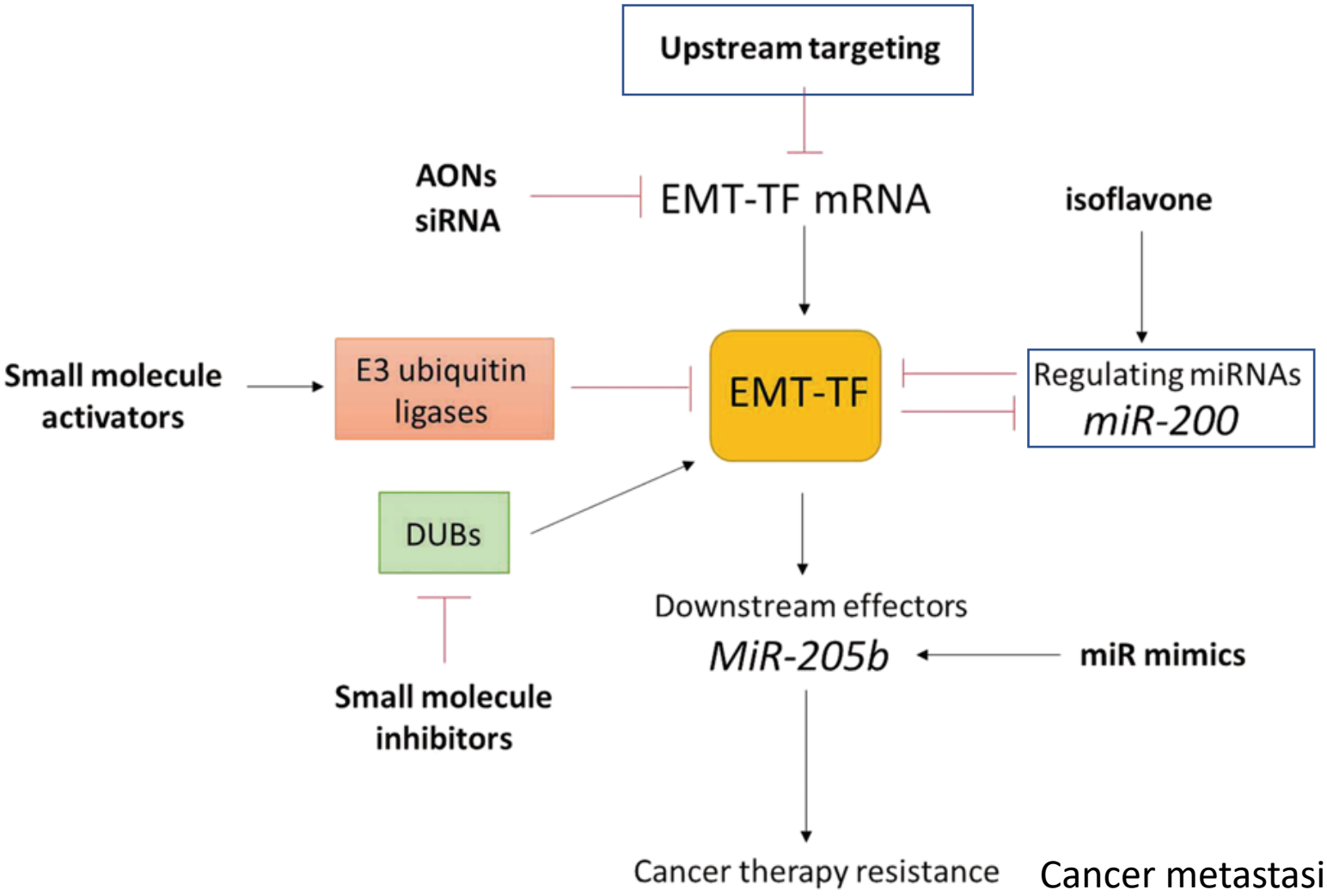
Epigenetic modifications
- DNA methylation
- Chromatin remodeling
- Histone code
- Regulating alternative splicing



S. Imani et al. Scientific Reports **volume6**, Article number: 28587 (2016)

Anti-cancer strategy toward EMT

・Targeting EMT-TFs or EMT-TFs signaling pathways (siRNA, miRNA, small inhibitor/activator)
・Reduction of EMT-TFs results in MET (partly)
・Combination therapy with chemotherapy



To prevent EMT of cancer cells, blocking TGFβ pathway seems to be promising.

Combination of αTGFβ + αPD-L1 provoked tumor regression.

LETTER

doi:10.1038/nature25501

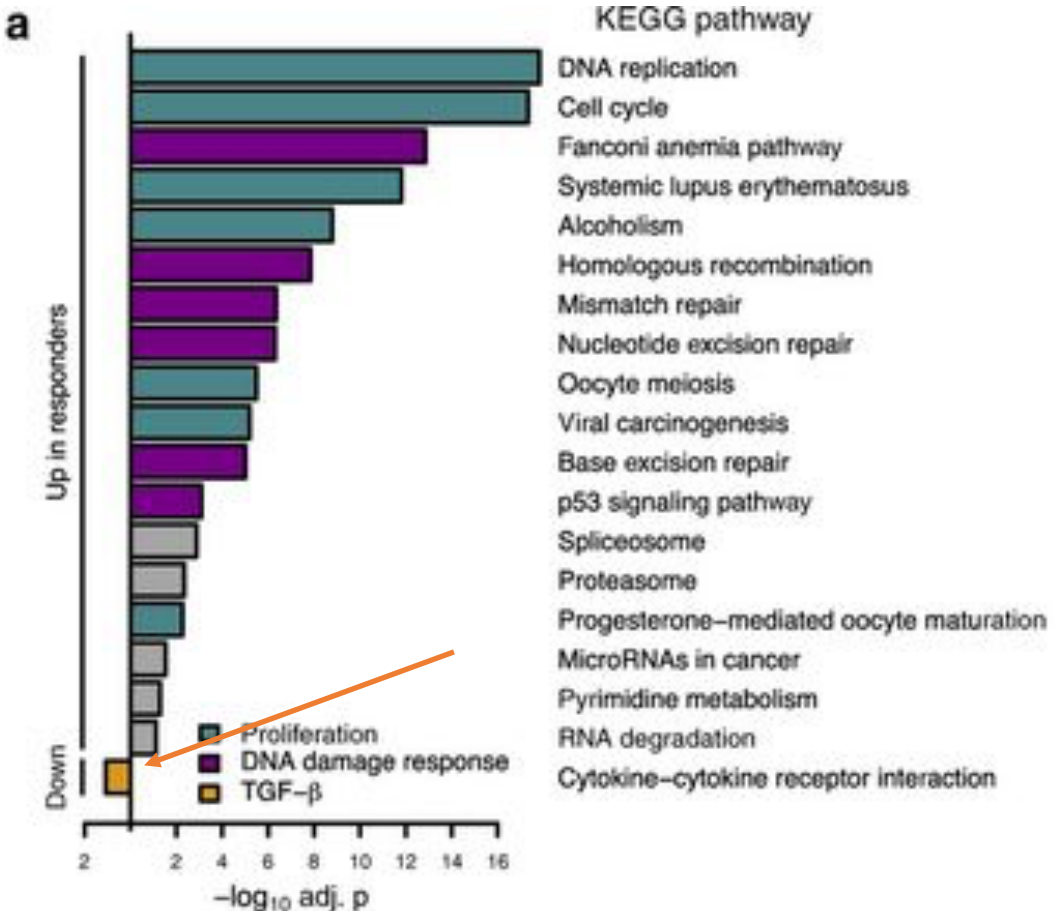
TGFβ attenuates tumour response to PD-L1 blockade by contributing to exclusion of T cells

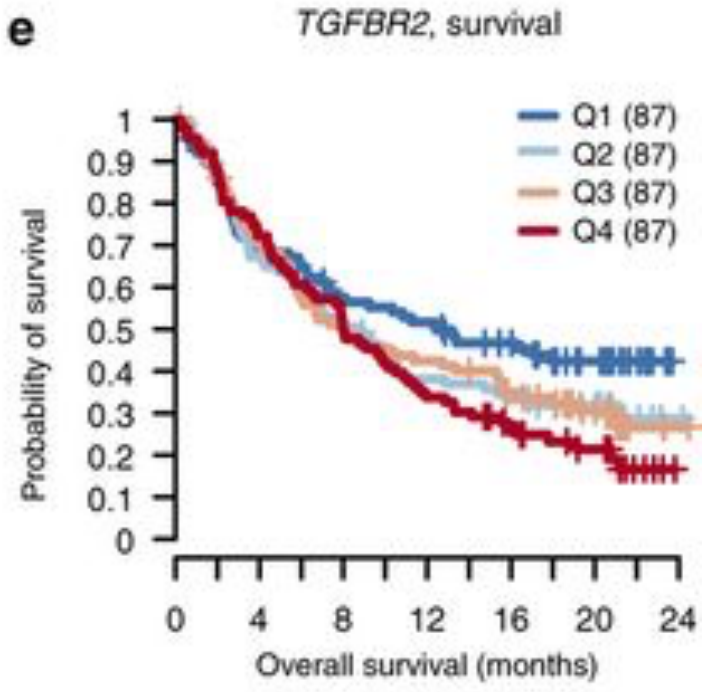
Sanjeev Mariathasan[1]*, Shannon J. Turley[1]*, Dorothee Nickles[1]*, Alessandra Castiglioni[1], Kobe Yuen[1], Yulei Wang[1], Edward E. Kadel III[1], Hartmut Koeppen[1], Jillian L. Astarita[1], Rafael Cubas[1], Suchit Jhunjhunwala[1], Romain Banchereau[1], Yagai Yang[1], Yinghui Guan[1], Cecile Chalouni[1], James Ziai[1], Yasin Şenbabaoğlu[1], Stephen Santoro[1], Daniel Sheinson[1], Jeffrey Hung[1], Jennifer M. Giltnane[1], Andrew A. Pierce[1], Kathryn Mesh[1], Steve Lianoglou[1], Johannes Riegler[1], Richard A. D. Carano[1], Pontus Eriksson[2], Mattias Höglund[2], Loan Somarriba[3], Daniel L. Halligan[3], Michiel S. van der Heijden[4], Yohann Loriot[5], Jonathan E. Rosenberg[6], Lawrence Fong[7], Ira Mellman[1], Daniel S. Chen[1], Marjorie Green[1], Christina Derleth[1], Gregg D. Fine[1], Priti S. Hegde[1], Richard Bourgon[1] & Thomas Powles[8]

Lack of response to anti-PD-L1 was associated with a signature of TGF-β signaling in fibroblasts

(from a large cohort of patients of MUC treated with anti-PD-L1 (atezolizumab).

Pathways associated with response to atezoilzumab and cancer–immune phenotypes

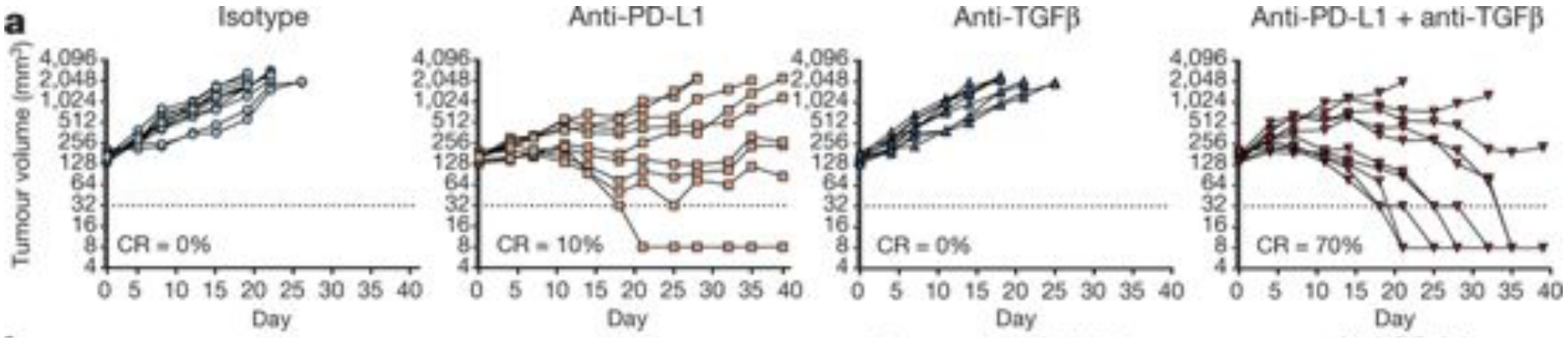


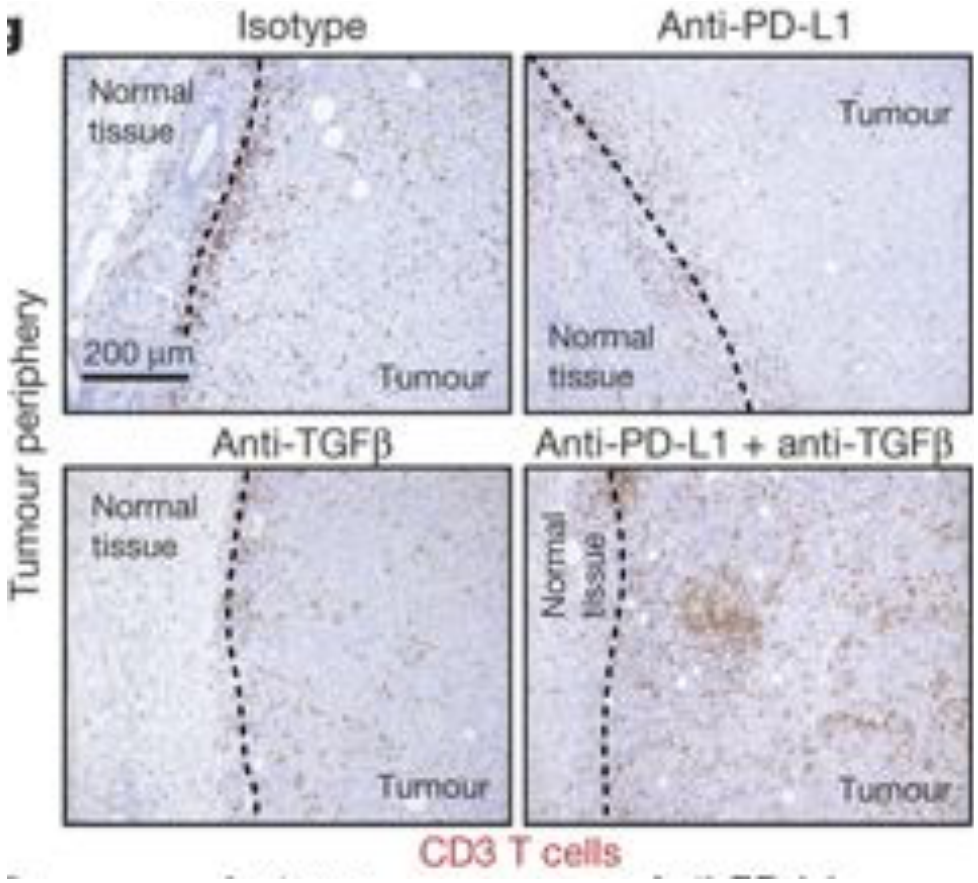


TGFBR2 gene expression in is significantly associated with non-response.

Nature vol554, 2018 544

Combination of αTGFβ + αPD-L1 provoked tumor regression.





EMT6 mouse mammary carcinoma model (immune-excluded phenotype)

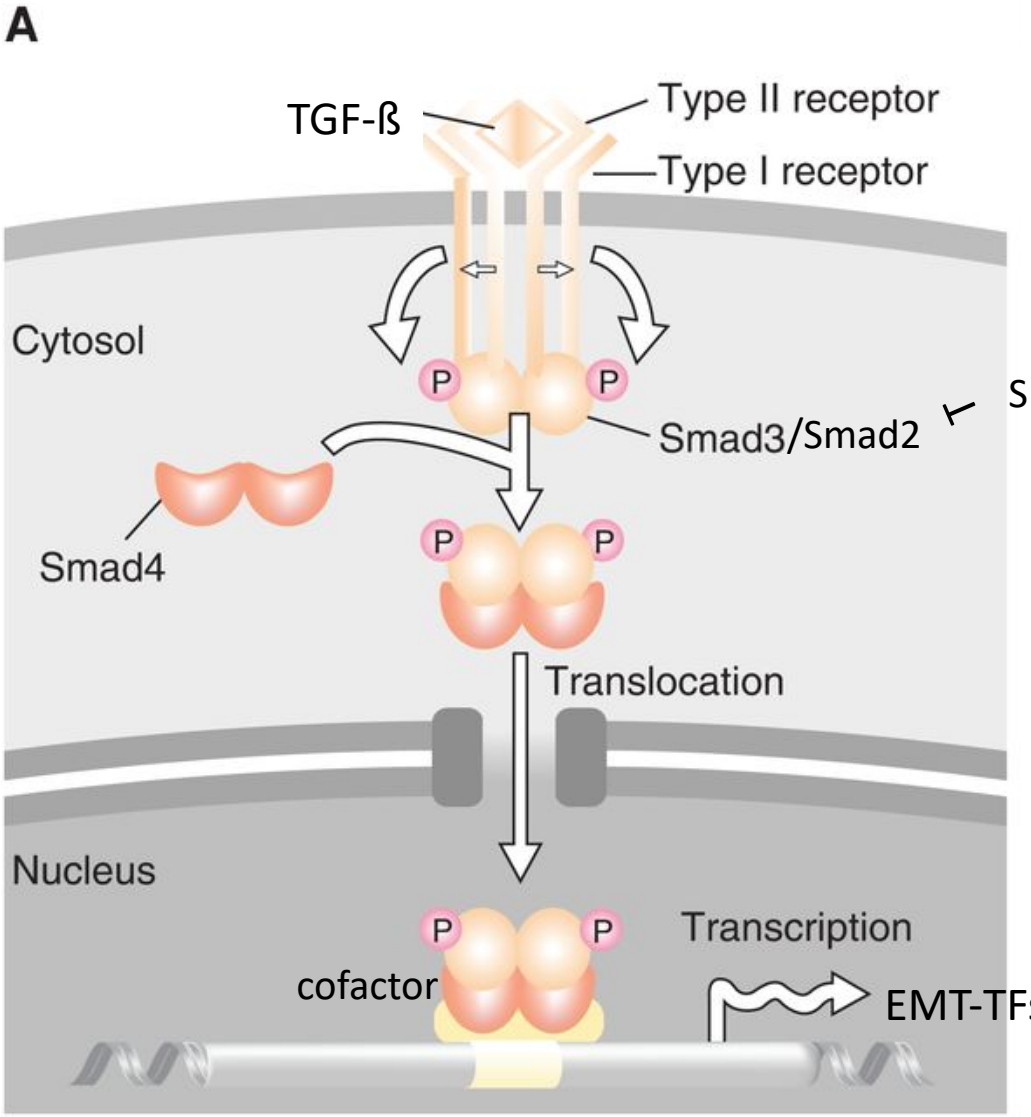
Anti-TGFβ treatment significantly reduced TGFβ receptor signaling (pSMAD2/3).

Combined antibody led increase in tumor-infiltrating T cells.

Nature vol554, 2018 544

Contents

TGF-β Signal ... one of the upstream pathways of EMT.



Transforming growth factor (TGF)-β

- Cell growth inhibitory function
- Induce EMT-TFs through SMADs signaling

SMAD7 ⊣

(ZEB1) ⊣

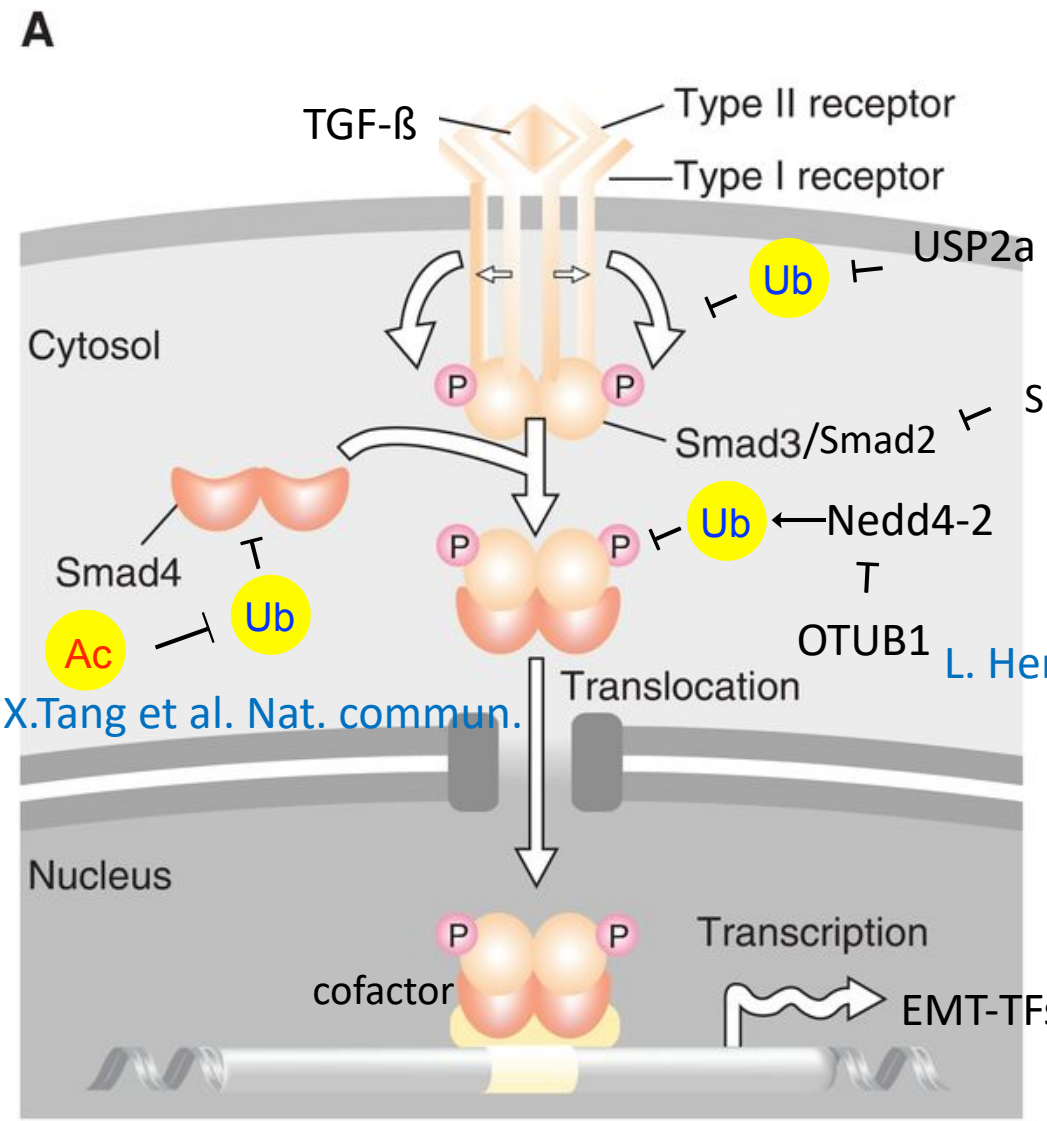
miRNA200 family inhibits transcription of EMT-TFs induced by TGFβ.

P. A. Gregory et. al. Nature cell biology, 10, 593, 2008.

Moses H.L. et al. Cold Spring Harb. Perspect. Biol. 8, a021865

TGF-β Signal is regulated by ubiquitin

TGF-β–SMAD signal path is maintained by many ubiquitination.



Zhao et al. 2018, Cell Reports 22, 2442.

Z. Zhang et al. 2017, 8, 2116

L. Herhaus et al. Nature commun. 2013, 4, 2519

X.Tang et al. Nat. commun.

miRNA200 family

(ZEB1)

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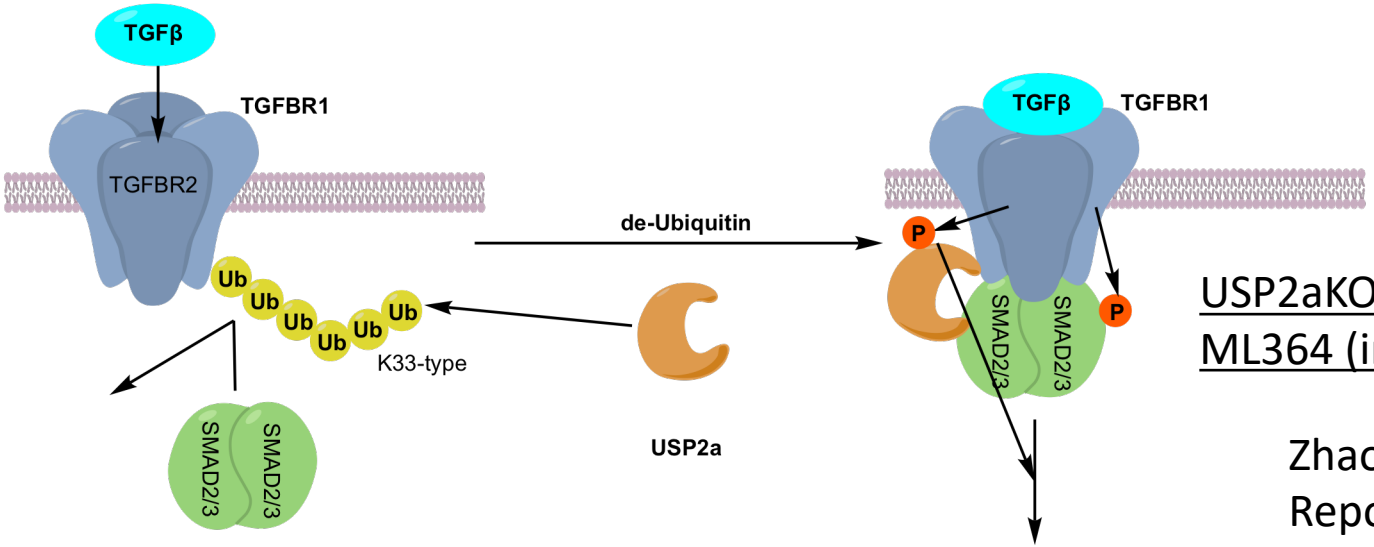
Role of Ubiquitin in inhibition of the TGFβ signaling

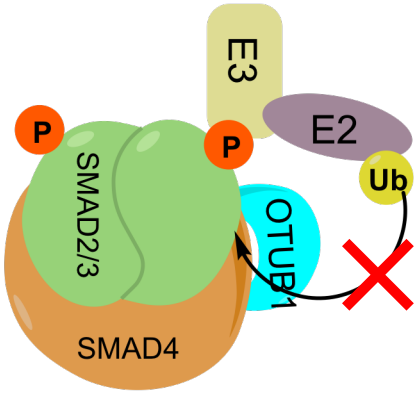
・Steric hinderance (K33-type)



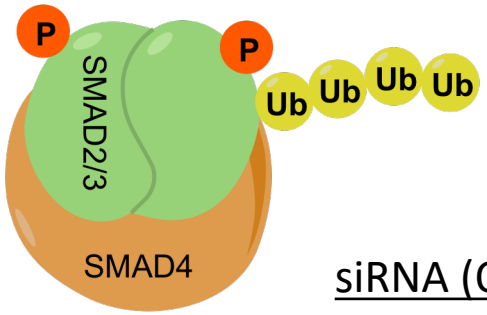
USP2aKO Hep3B injected mice or ML364 (inhibitor) to injected mice

Zhao et al. 2018, Cell Reports 22, 2442.

・Degradation (K48-type)



Nuclear importation
EMT-TFs transcription

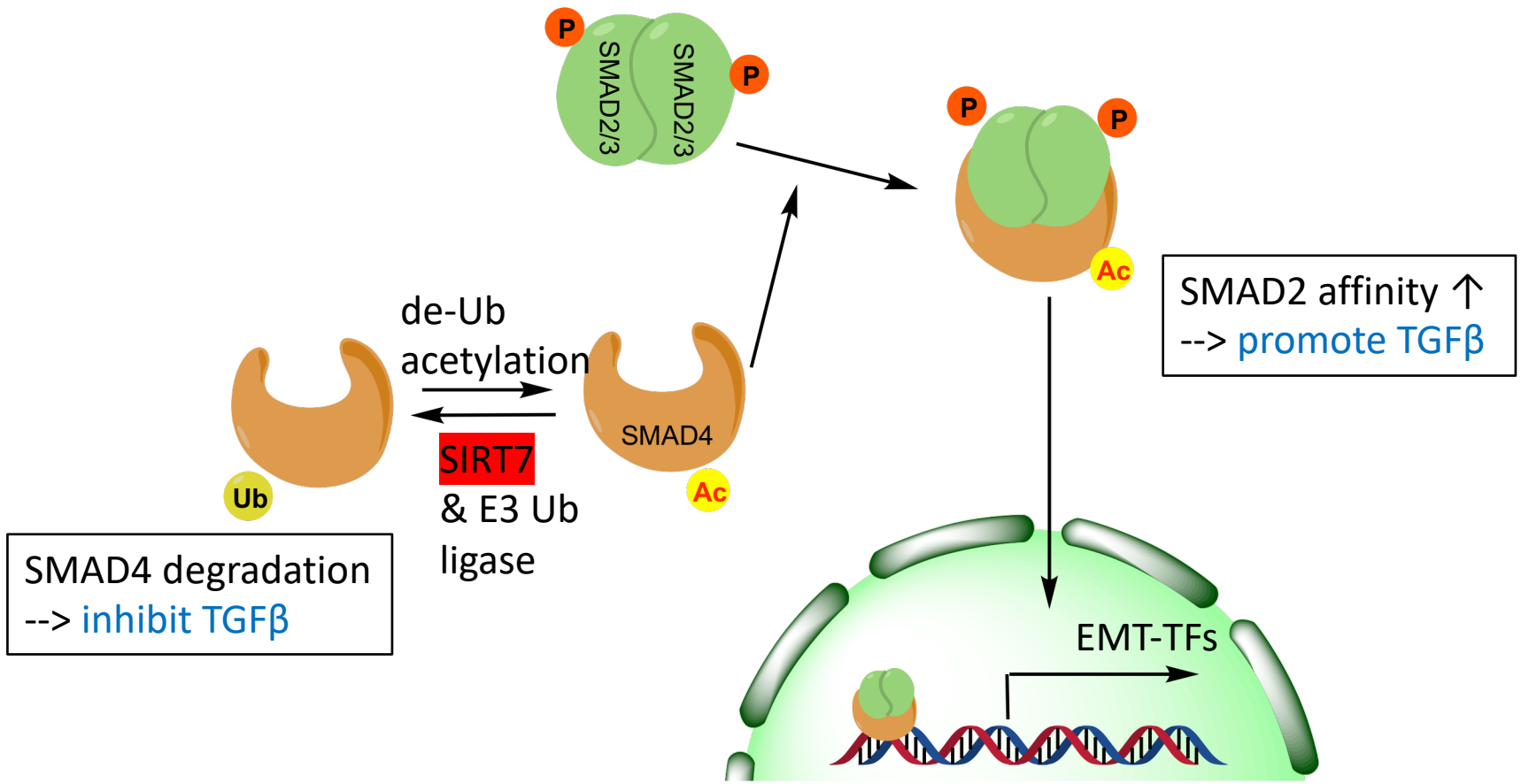


degradation by proteasome
TGFβ signal is stopped

siRNA (OTUB1) inhibits cell migration

L. Herhaus et al.
Nature commun. 2013, 4, 2519

SIRT7 antagonizes TGF-β signaling and inhibits breast cancer metastasis

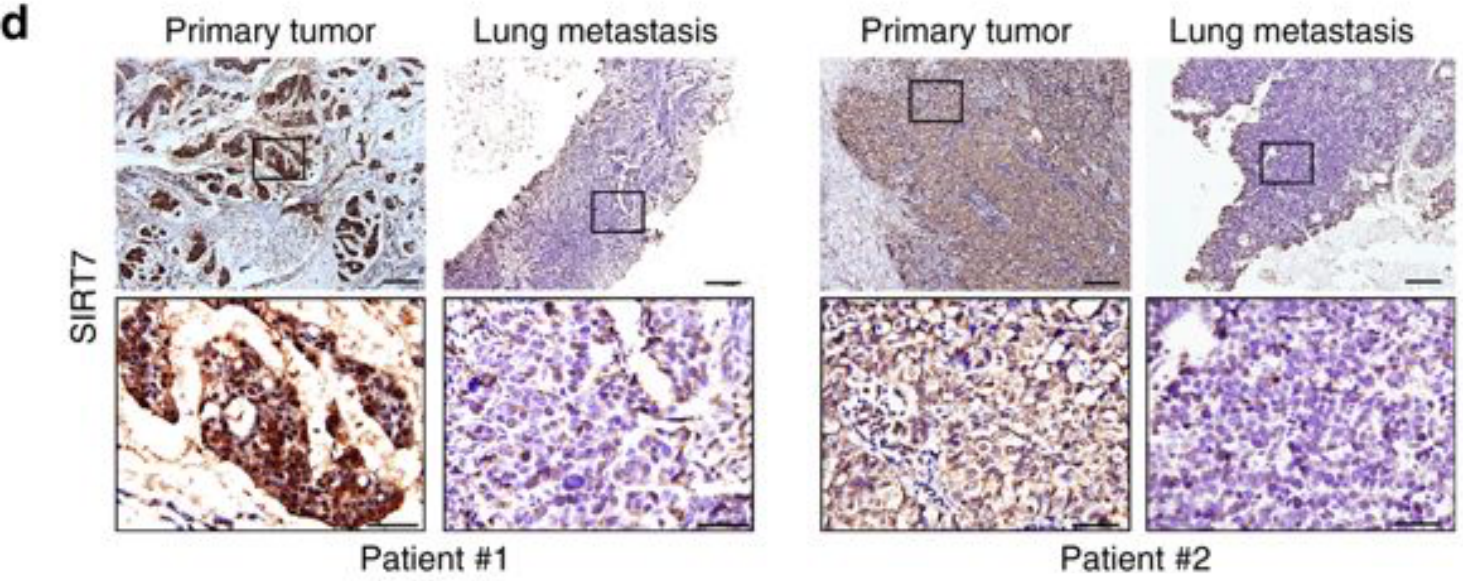


Resveratrol activates SIRT7 and inhibit TGFβ, metastasis is inhibited in model mouse.

X.Tang et al. Nat. commun. 8:318 (2017)

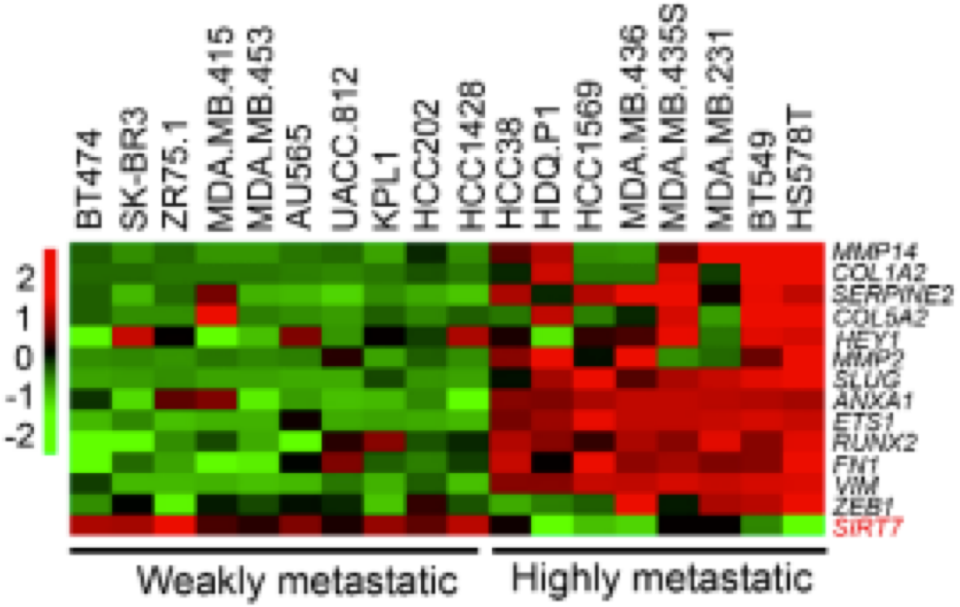
SIRT7 is downregulated in breast cancer lung metastasis

brown : IHC staining of SIRT7



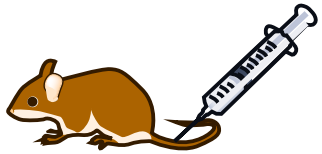
・SIRT7 was downregulated in lung metastasis compared to in primary tumors.

・SIRT7 level is inversely correlated with metastatic capacity of various breast cancer cell lines.



X.Tang et al. Nat. commun. 8:318 (2017)

SIRT7 inhibits breast cancer lung metastasis



4T1-shSirt7 cells

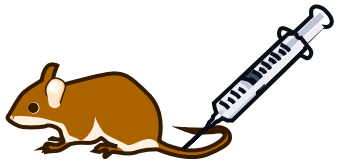
4 weeks

Balb/c mice

4T1-shSirt7 cells

4 weeks

SIRT7 KD promotes lung metastasis.



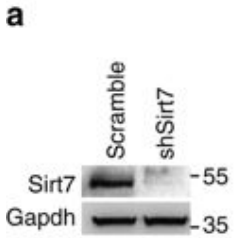
MDA-MB231 cells over expressing SIRT7

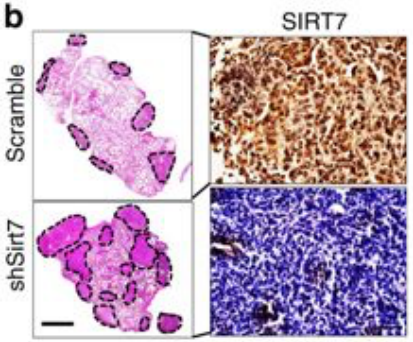
4 weeks

MDA-MB231 cells over expressing SIRT7

4 weeks

SIRT7 over expressing prevents lung metastasis.

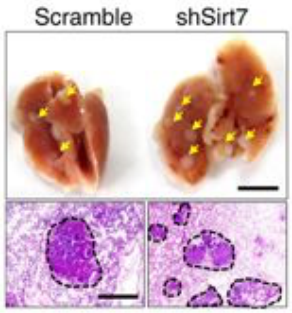


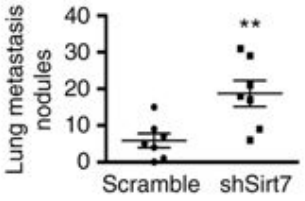


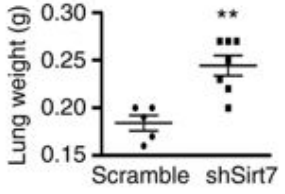
Knockdown of *Sirt7* promotes breast cancer metastasis to lung

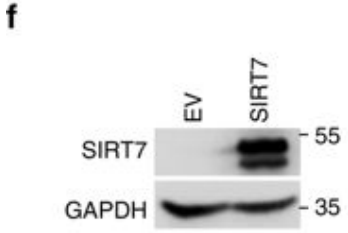
4T1 cells	Metastatic incidence	Metastatic nodules
Scr	3/6	148
shSirt7	6/6	564

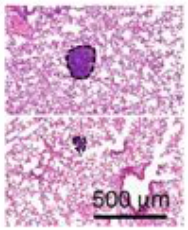
Note: tail vein injection, $p<0.05$, by non-parametric Mann–Whitney *U*-test)

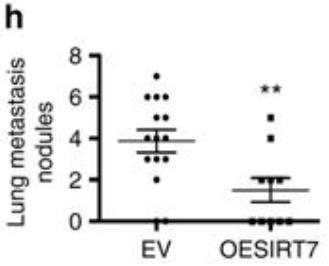


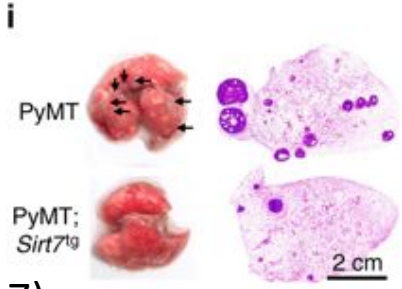


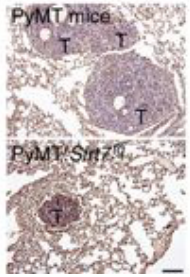


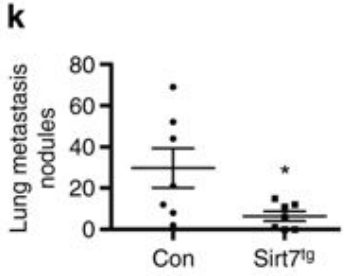








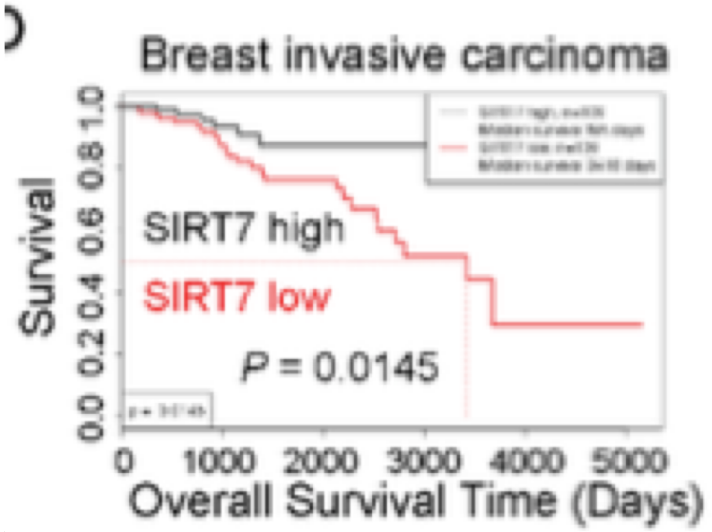




X.Tang et al. Nat. commun. 8:318 (2017)

SIRT7 expression is associated with TGF-β signaling

KEGG* analysis in TCGA**-RNA-seq deta set obtained from 1097 individuals



*Kyoto Encyclopedia of Genes and Genomes
Database for bioinformatics, based on molecular interaction network (signal path, metabolism...)

**Database of The Cancer Genome Atlas
(National Cancer Institute &
National Human Genome Research Institute)

a SIRT7 negatively associated genes

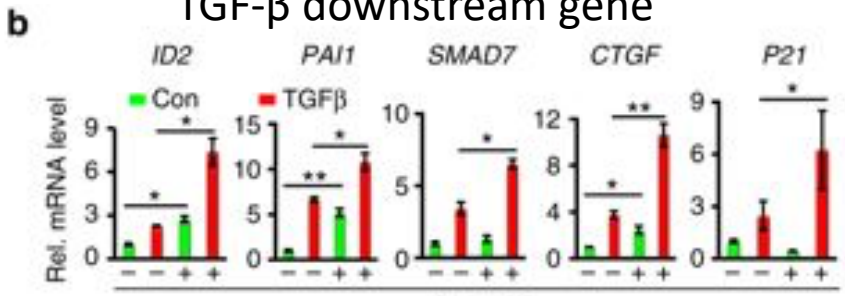
Pathway	p.geomean	stat.mean	p.val	q.val	set.size
hsa04510 Focal adhesion	**0.00011**	**-3.83625**	**1E-04**	**0.01**	**55**
hsa04512 ECM-receptor interaction	**0.00035**	**-3.68957**	**4E-04**	**0.01**	**28**
hsa04972 Pancreatic secretion	0.00177	-3.39427	0.0018	0.035	15
hsa04630 Jak-STAT signaling pathway	0.00221	-3.00039	0.0022	0.035	27
hsa04020 Calcium signaling pathway	0.00254	-2.8989	0.0025	0.035	36
hsa04360 Axon guidance	0.00306	-2.84102	0.0031	0.035	32
hsa04350 TGF-beta signaling pathway	**0.00356**	**-2.90561**	**0.004**	**0.04**	**21**
hsa04810 Regulation of actin cytoskeleton	0.00406	-2.70267	0.0041	0.035	52
hsa04270 Vascular smooth muscle contraction	0.00698	-2.53492	0.007	0.054	31
hsa02010 ABC transporters	0.01495	-2.34241	0.015	0.103	13
hsa04540 Gap junction	0.03588	-1.84057	0.0359	0.201	26
hsa04974 Protein digestion and absorption	0.03743	-1.83086	0.0374	0.201	21
hsa00510 N-Glycan biosynthesis	0.03864	-1.87511	0.0386	0.201	10
hsa00562 Inositol phosphate metabolism	0.04081	-1.82046	0.0408	0.201	14
hsa04310 Wnt signaling pathway	0.04824	-1.69809	0.0482	0.215	24
hsa04970 Salivary secretion	0.04995	-1.702	0.05	0.215	15

*Extra Cellular Matrix

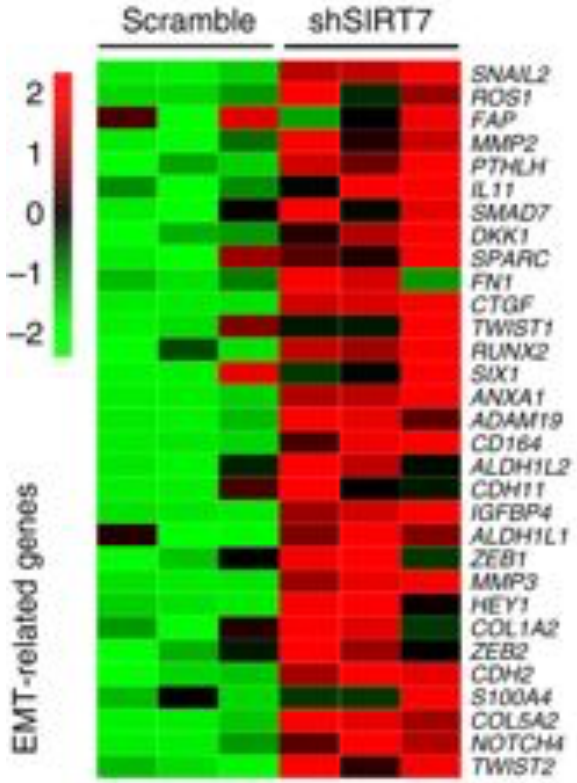
SIRT7 is important for TGFβ signal ??

SIRT7 indeed inhibits TGF-β signaling and EMT.

TGF-β downstream gene

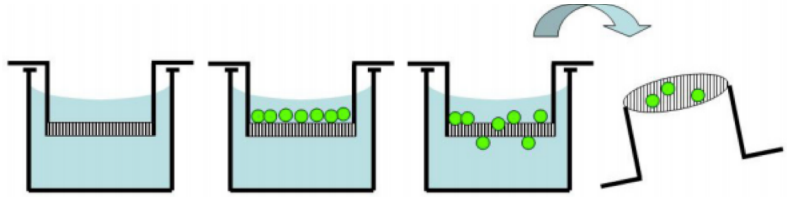


(Knock Down) si-SIRT7



Genes which mediate EMT is enriched by SIRT7 KD.

<Trans well assay (cell invasion)>



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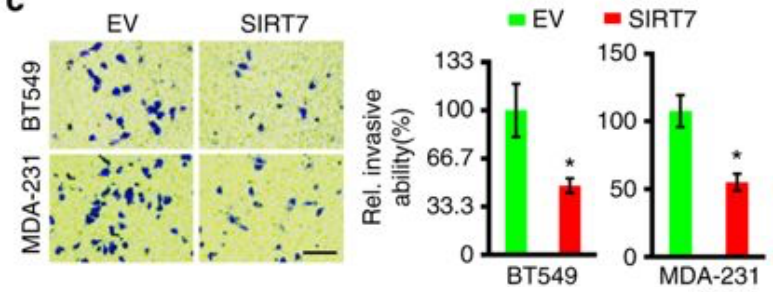
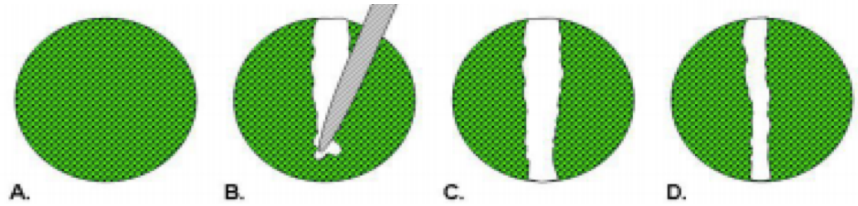
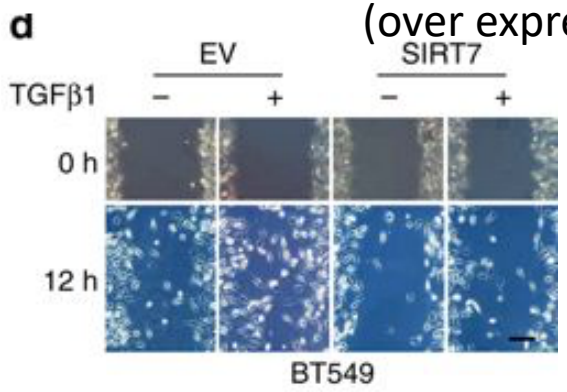
(over expression)



<Trans well assay (cell migration)>



(over expression)



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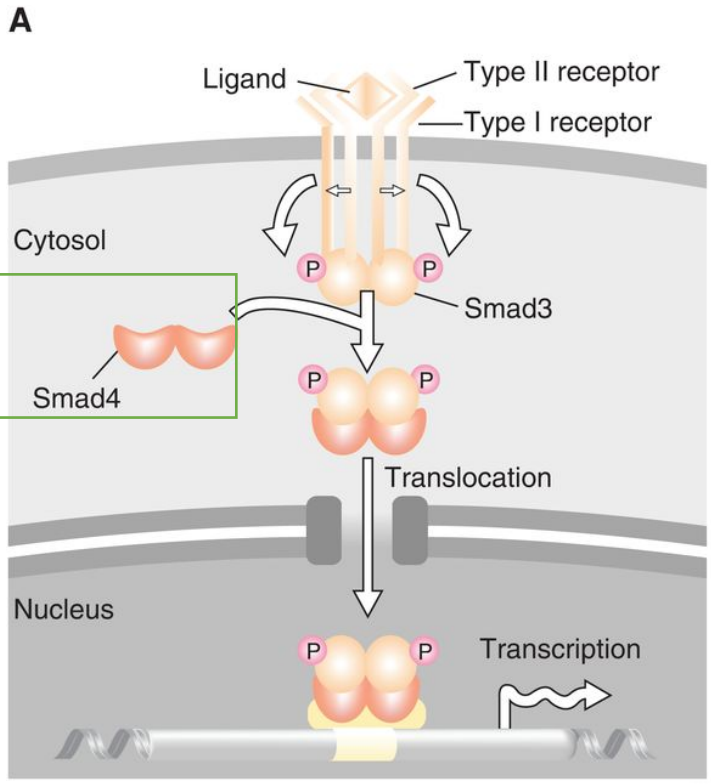
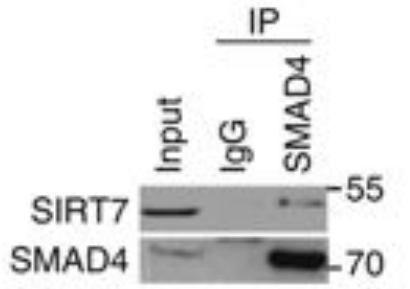
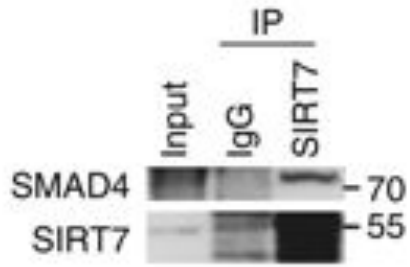
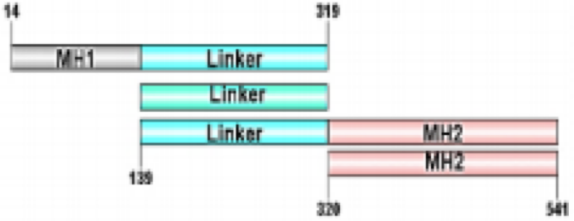
SIRT7 interacts with SMAD4, especially SMAD4(MH2)-SIRT7(Catalytic domain).

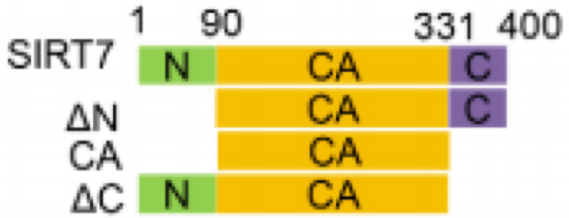


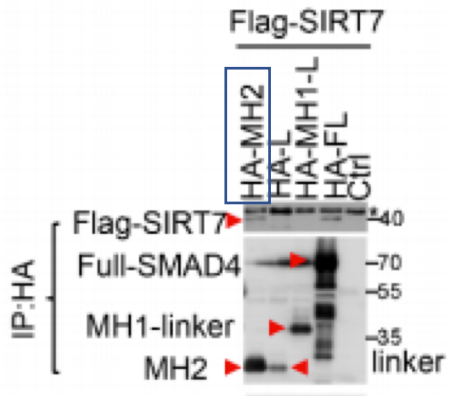


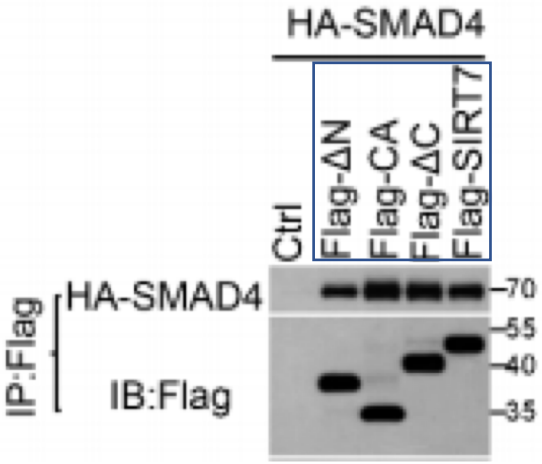


SMAD4

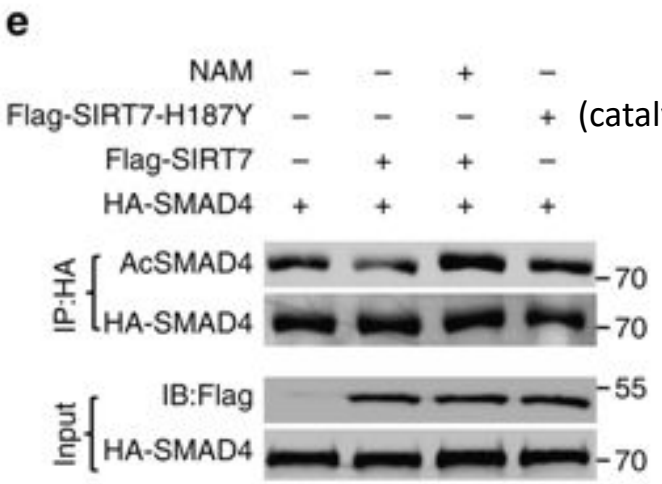




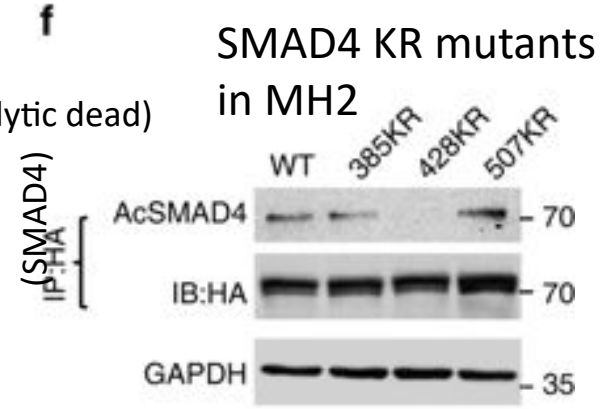




SIRT7 deacetylates SMAD4(K428)

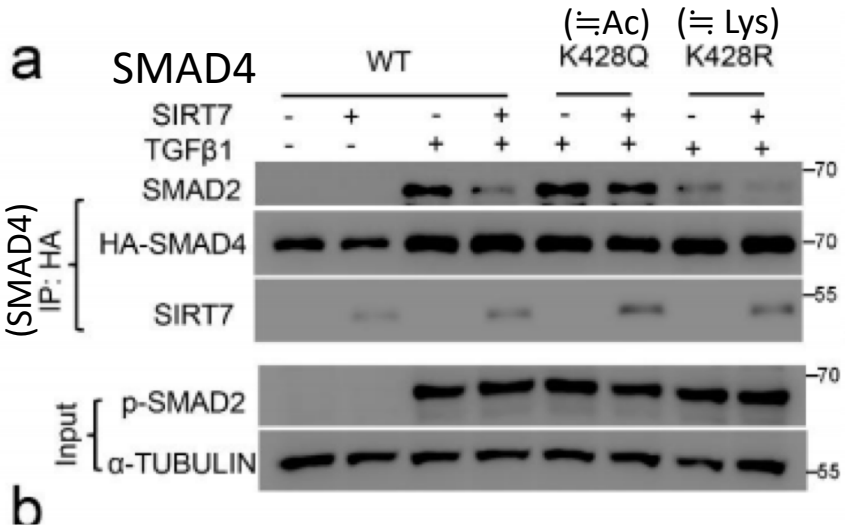


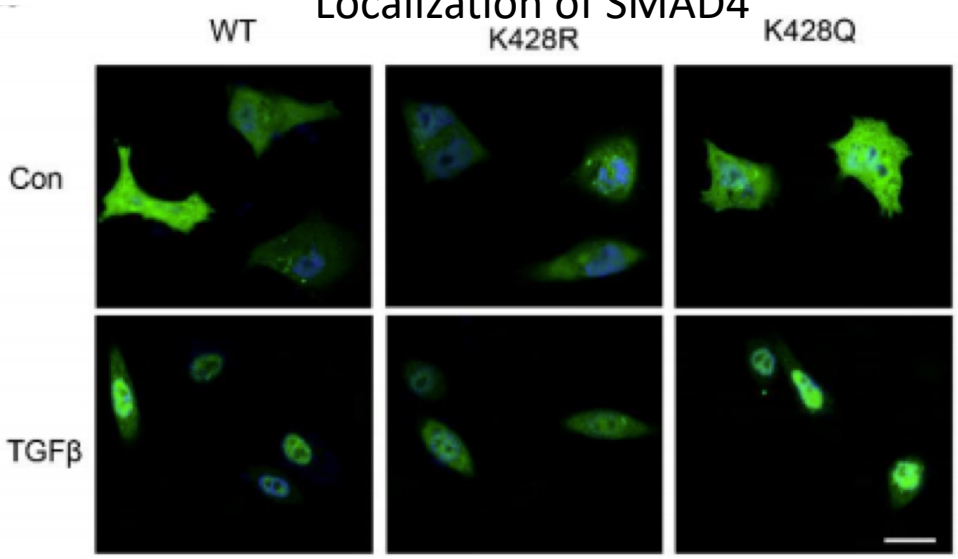
(catalytic dead)



SMAD4 KR mutants in MH2

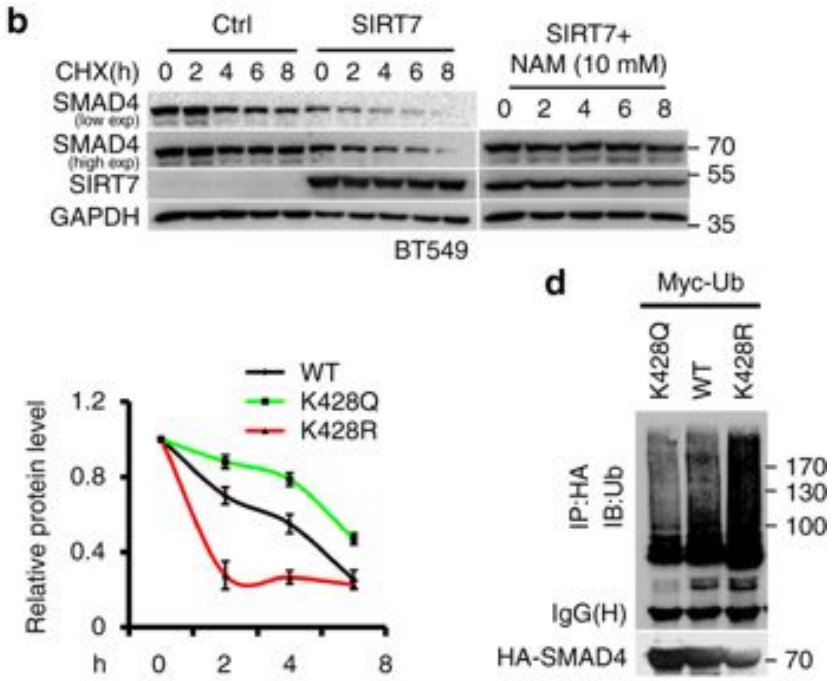
SMAD4(K428Ac) is important for SMAD2-SMAD4 binding, nuclear localization by TGF-β siganal.





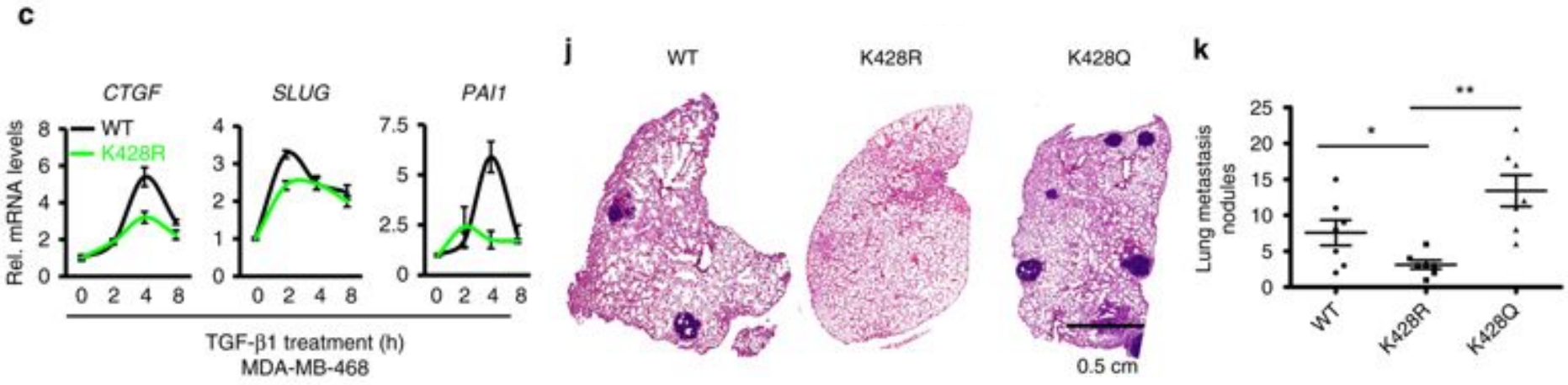
weak signal
weak localization

SMAD4(K428) is important for its degradation (Ubiquitination)

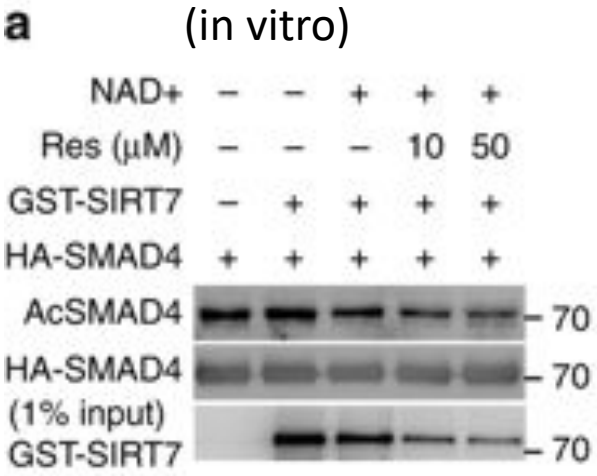


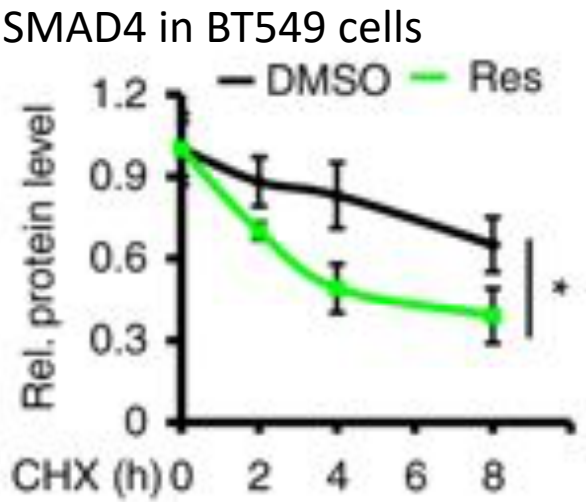
SMADK428	(TGFβ)
Ac(Q) ...	SMAD2 binding ↑ (↑)
	Ub degradation ↓ (↑)
K (R) ...	SMAD2 binding ↓ (↓)
(SIRT7)	Ub degradation ↑ (↓)

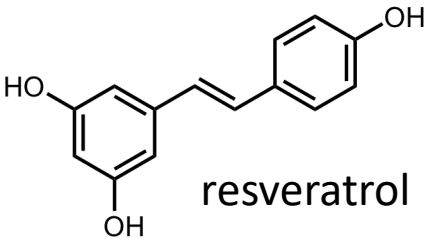
SMAD4 (Ac) is important for TGFβ-induced metastasis



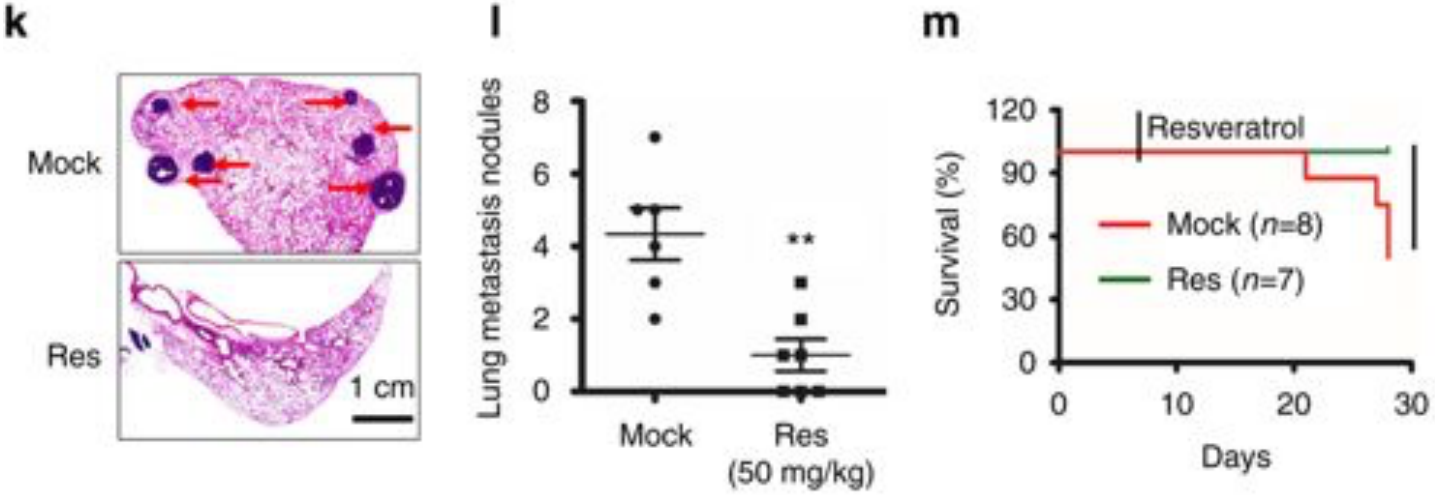
Resveratrol activates SIRT7 and inhibits lung metastasis.
(*Resveratrol is known as SIRT1 activator)







Bulb/c mice injected with 4T1 cells



Summary

・EMT is critical step for tumor metastasis, so blocking EMT can be a effective treatment for cancer metastasis.

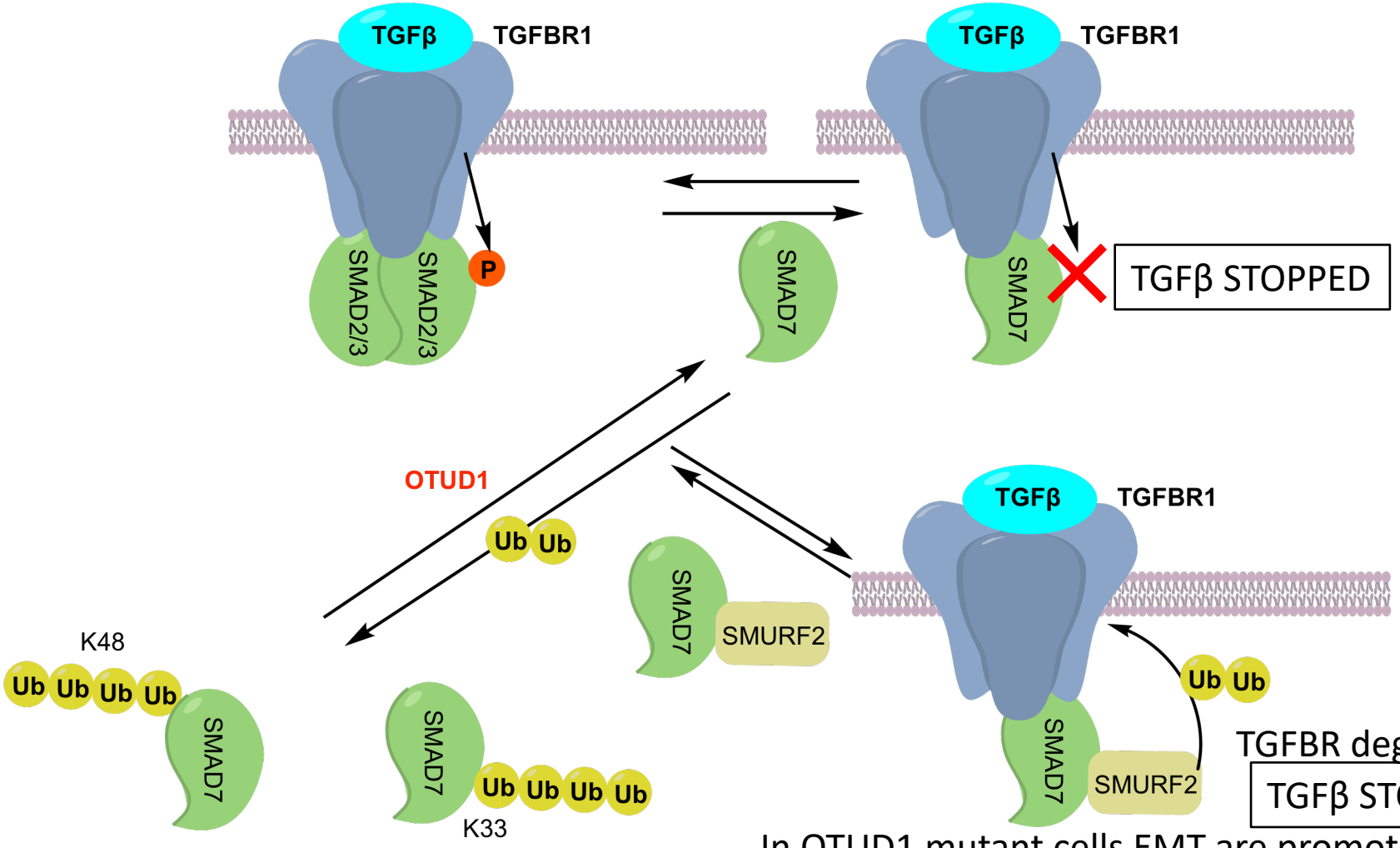
・EMT is induced by cellular crosstalk with extracellular environment, then intracellular environment is maintained (epigenetically).

・TGFβ signaling pathway is one of EMT-inducing pathways, and blocking TGFβ in combination with conventional anti-cancer treatment.

・TGFβ signaling is mediated by SMADs, which are regulated by Ubiquitination. These PTMs control activity or stability of SMADs.

Role of Ubiquitin in inhibition of the TGFβ signaling

- Steric hinderance (K33-type)
- Degradation (K48-type)



SMAD7 degradation

TGFβ Promoted

inhibition of SMURF2 binding

In OTUD1 mutant cells EMT are promoted injected mice

Z. Zhang et al. Nat. commun. 8:2116 (2017)