

The roles of thrombospondin-1 in tumor angiogenesis and stroma reaction during cervical carcinogenesis

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The cancer invasion process is associated with stroma reaction

- ‘Seed and soil hypothesis’ of cancer biology,
 - the ‘seeds (cancer cells)’ must find a receptive ‘soil (environment)’ (Paget S 1889 Lancet, Fidler IJ 2003 Nat Rev Cancer).
- The normal host microenvironment is nonpermissive for neoplastic progression,
 - c.f. tumor-reactive stroma promotes neoplastic growth and metastasis (Chlenski A et al. 2007 Oncogene).

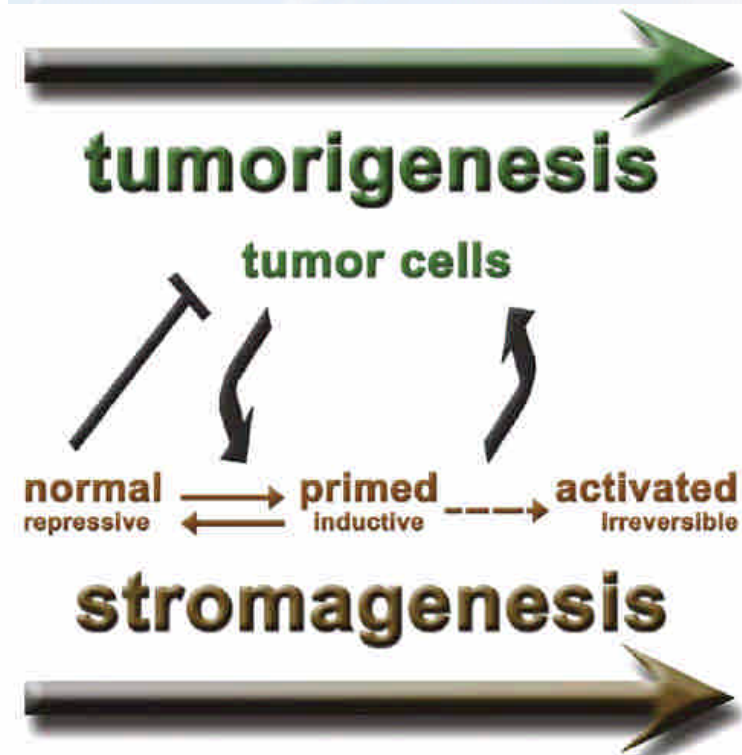


Paget S 1889 Lancet

Stroma reaction (stromagenesis)

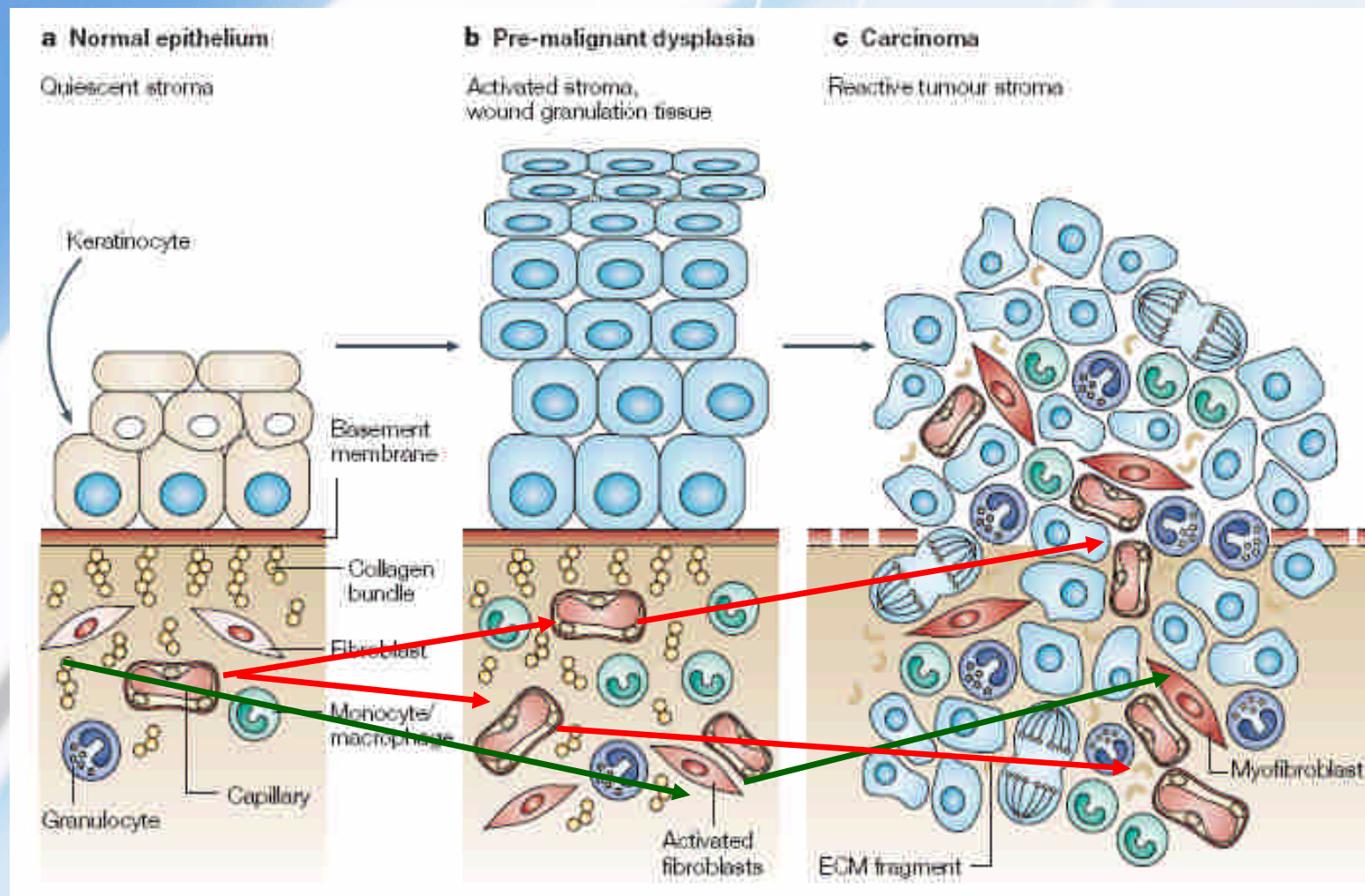
- Tumor microenvironment can significantly influence tumorigenesis (Mueller MM & Fusenig NE 2004 Nat Rev Cancer)
- **Stroma reaction**, also known as stromagenesis
 - A host reaction of mesenchymal alteration
 - Occurs parallel to tumorigenesis
 - Produces a progressive and permissive microenvironment
 - Supports tumor progression

(Amatangelo MD et al. 2005 Am J Pathol)



(Beacham DA & Cukierman E
2005 Semin Cancer Biol)

Stroma is an active participant in tumor progression



Reciprocal interactions between tumor cell and stroma

- A dynamically evolving microenvironment that fosters tumor cell invasion, survival and growth.

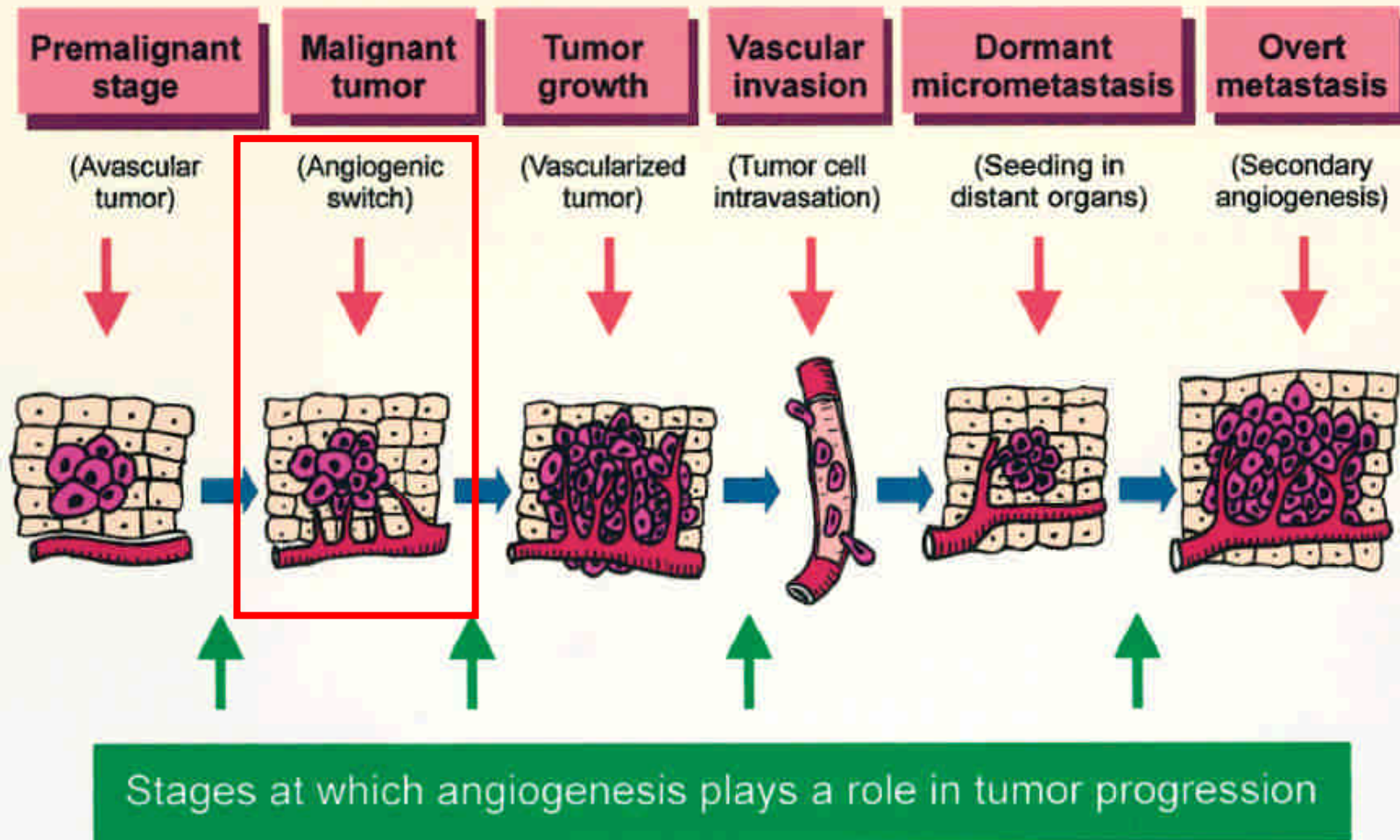
(1) **endothelial cells:** angiogenesis 

(2) **fibroblasts**

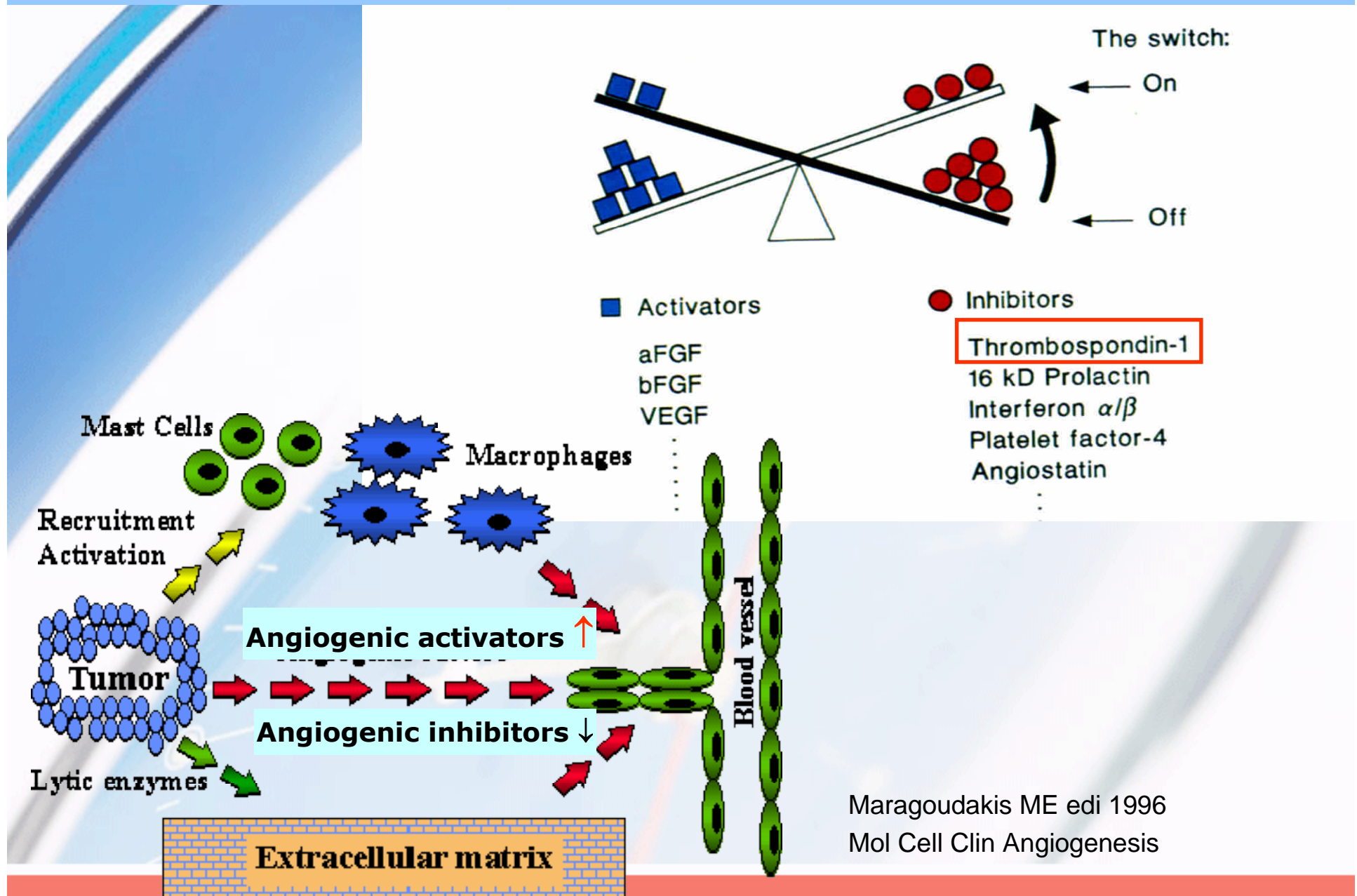
- **fibroblast** proliferation and trans-differentiation
- extracellular matrix (ECM) deposition and remodeling
- **stroma marker overexpression** (α -SMA, desmin, tenascin-C)
- increased matrix metallo-proteinase (MMPs) expression and activity

(3) **macrophages:** infiltration of immune cells

Angiogenesis participates in cancer development, growth, and metastasis



The balance hypothesis for the angiogenic switch



Maragoudakis ME edi 1996
Mol Cell Clin Angiogenesis

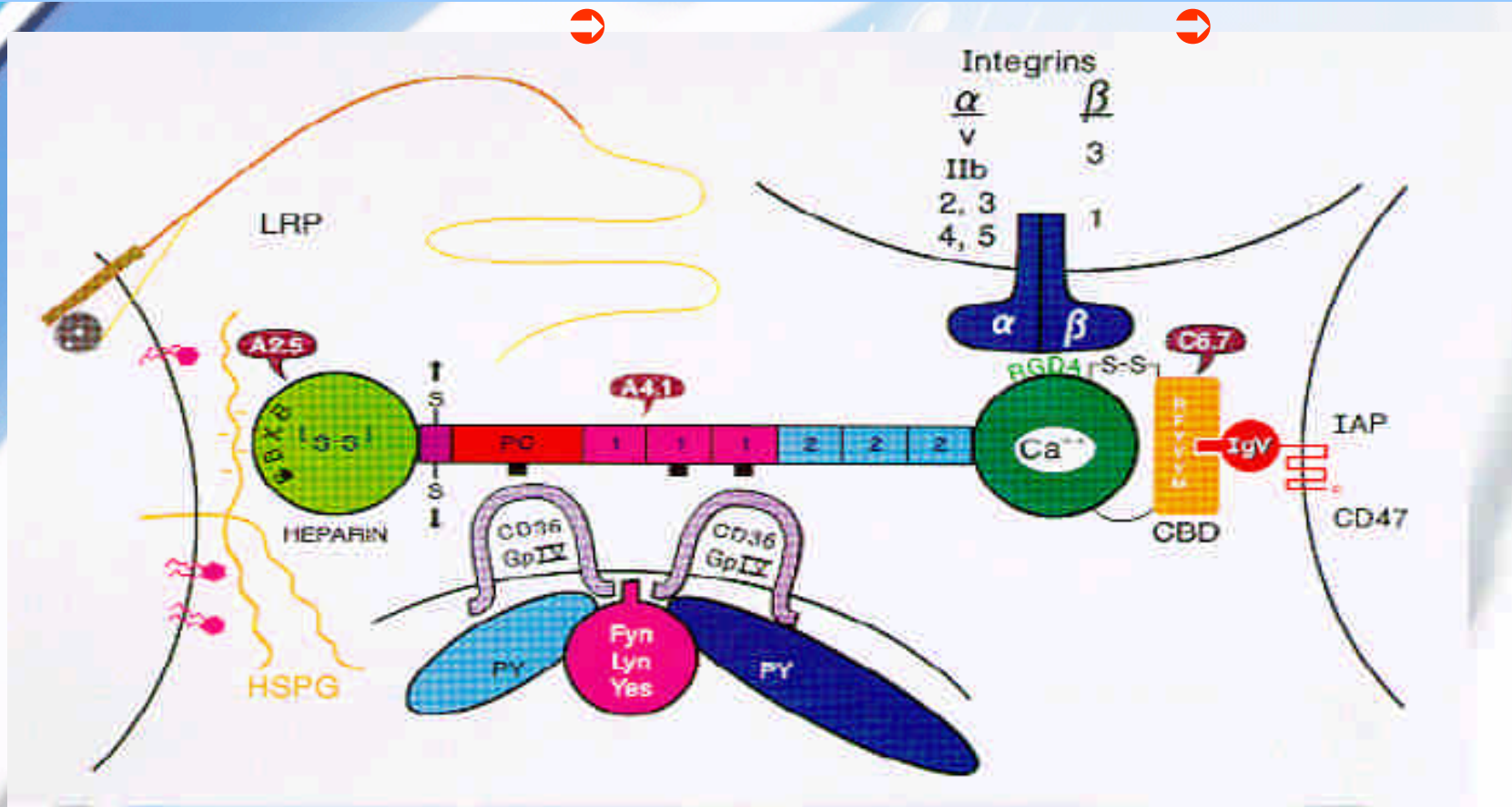
Background information

Thrombospondin 1 (TSP-1)

(Acc No. 188060) Gene map locus [15q15](#)

- a multi-modular secreted protein
 - associates with the extracellular matrix
 - possesses a variety of biologic functions, including a potent **angiogenic** activity.
- homo-trimeric glycoprotein with disulfide-linked subunits of MW 180,000.
- a component of the α -granule of **platelets**, released on platelet **activation**.
- synthesized and secreted for incorporation into the extracellular matrix
 - by a variety of cells, e.g. endothelial cells, fibroblasts, smooth muscle cells, and type II pneumocytes.

The structure of thrombospondin-1 (TSP-1) subunits and its receptors



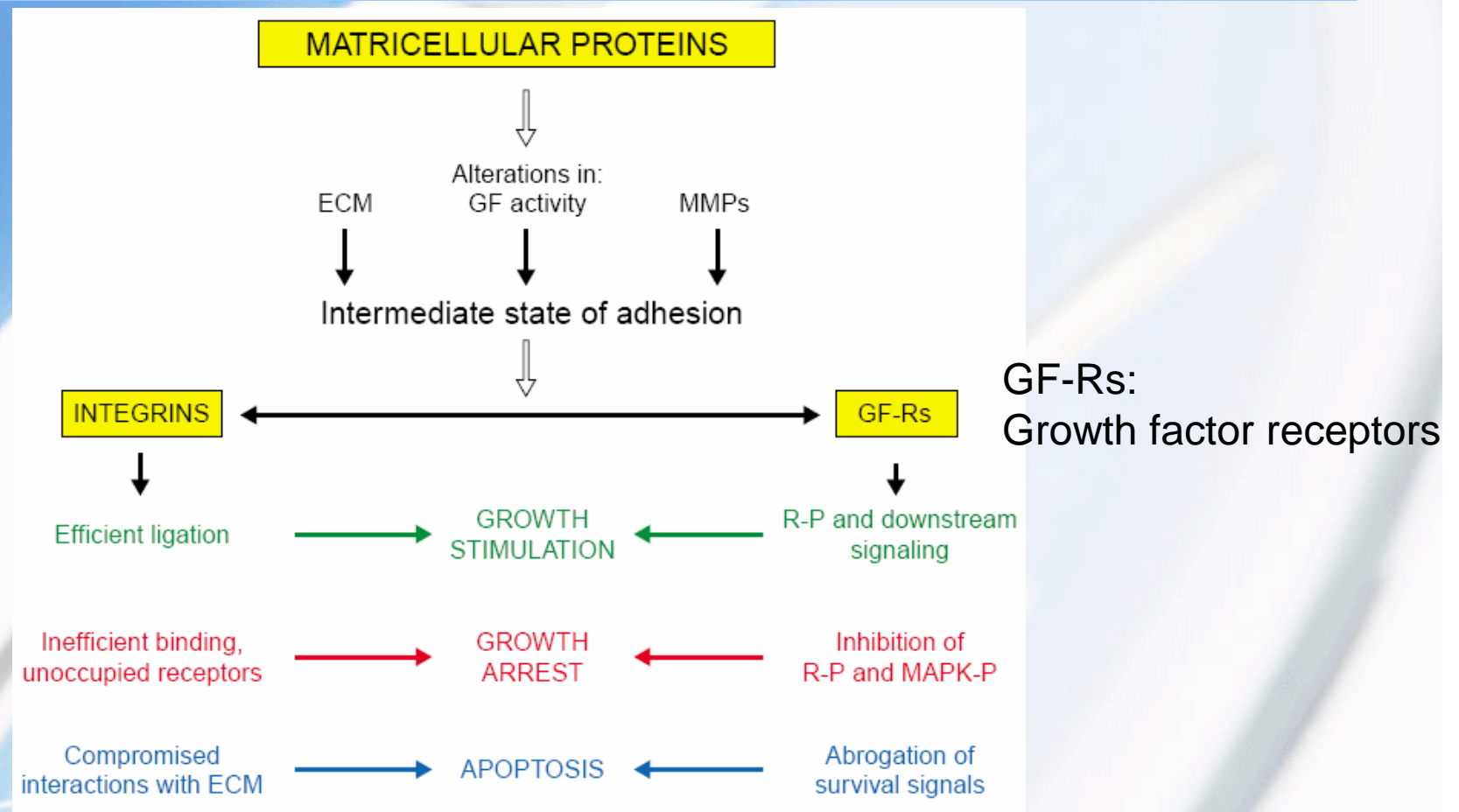
HBD: heparin-binding domain; PC: procollagens; Ca: Ca-binding domain;
 CBD: C-terminal binding domain ; IAP/CD47: integrin-associated protein
 LRP: LDL receptor-related protein; HSPG: heparan sulfate proteoglycans

Frazier WA, Washington University, St. Louis, MO

TSP-1 and tumor progression

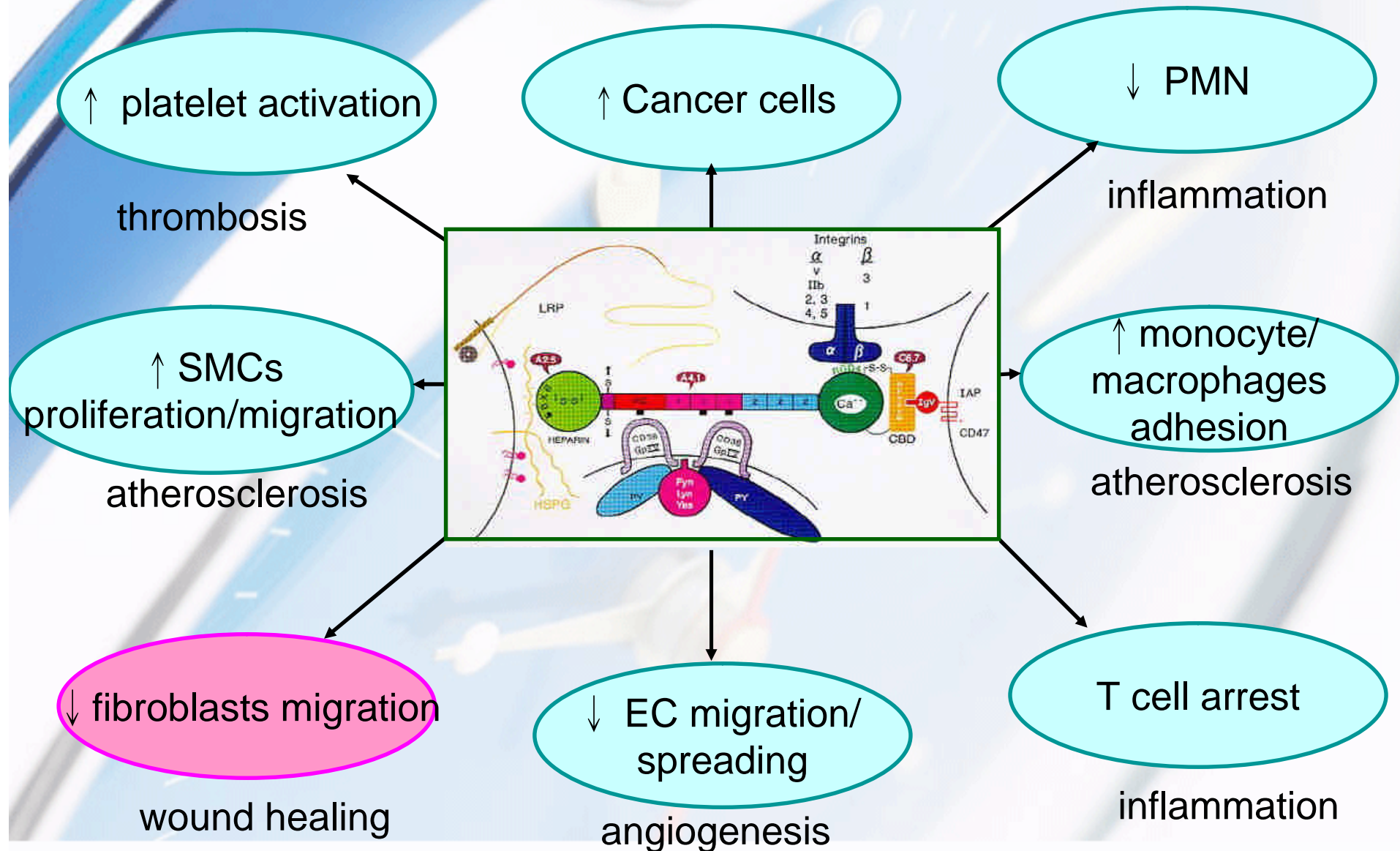
- One of the five members of TSPs →
 - **Matricellular protein** (Bornstein P 2000 J Clin Invest)
 - Cell-to cell and cell to-matrix communication
 - 450kDa homo-trimeric glycoprotein
 - With potent **anti-angiogenic** effects
- TSP-1 was reported to inhibit tumor growth by inhibiting angiogenesis
 - skin cancer (Streit M, et al. 1999 Am J Pathol)
 - prostate cancer (Jin RJ, et al. 2000 Cancer Gene Ther)
 - cervical cancer (Kodama J. 2001 Clin Cancer Res)
- The effects of TSP-1 in stroma reaction: unknown

Matricellular proteins affect receptors that mediate both adhesion and proliferation

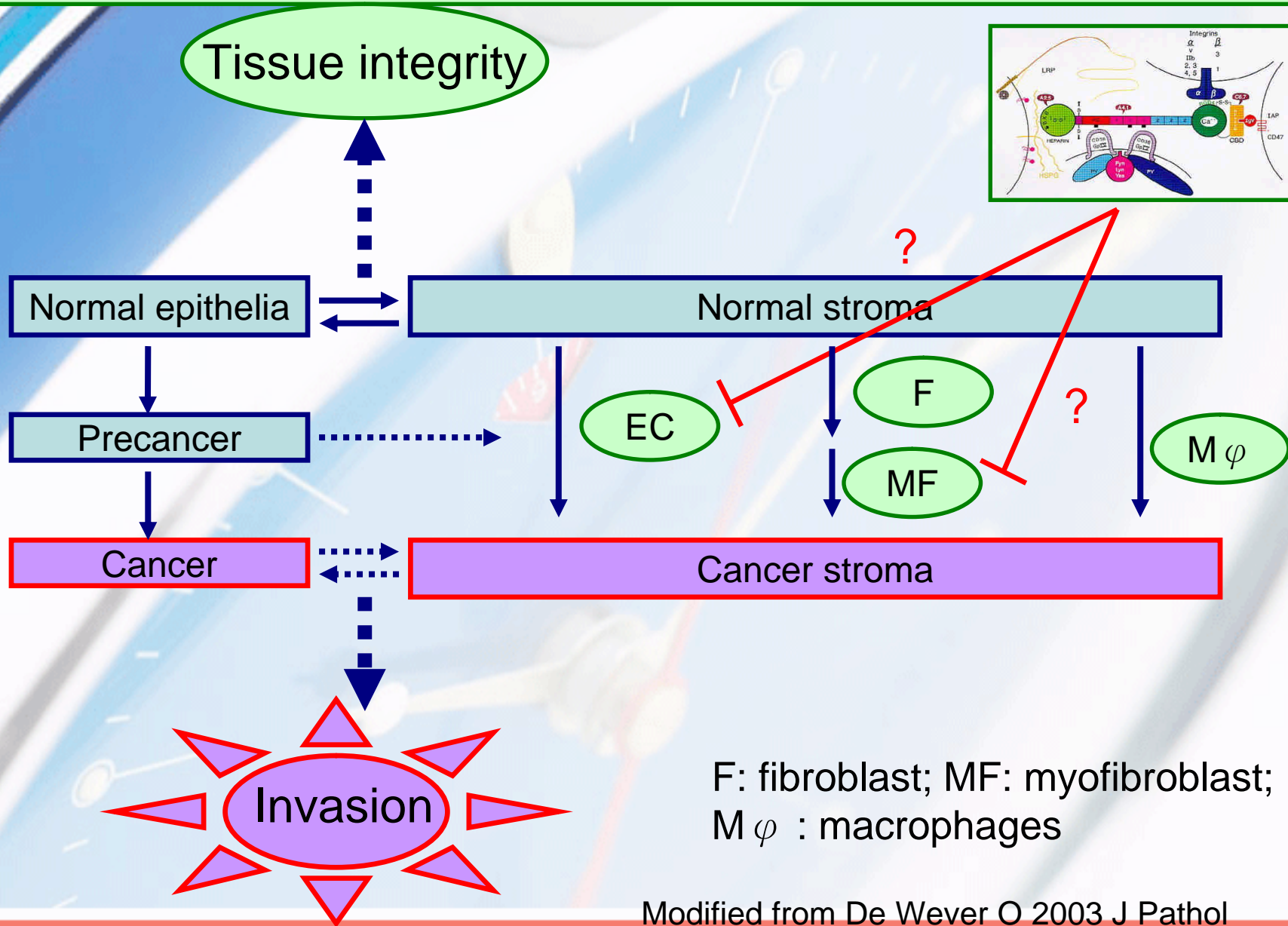


- Do not subserve structural roles but function contextually as modulators of cell-matrix interactions
- Bind to **many** cell-surface receptors, the ECM, growth factors, cytokines and proteases

The effects of TSP-1 on tumor and surrounding stromal cells

























Hypothesis: TSP-1 could inhibit cancer progression via angiogenesis (EC) and stroma reaction (F)



NORMAL



	Brain	
	Lung	No Data
	Heart	No Data
	Breast	
	Stomach	
	Pancreas	
	Liver	No Data
	Kidney	No Data
	Colon	
	Peritoneum	
	Ovary	
	Prostate	
	White Blood Cells	No Data
	Muscle	No Data



CANCER

TSP-1



SAGE anatomic viewer (CGAP)

The CANCER GENOME ANATOMY PROJECT

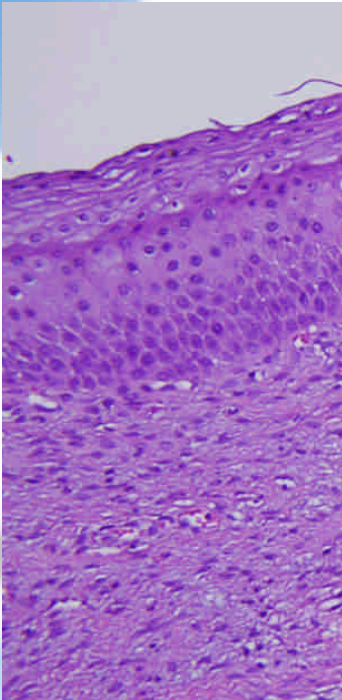
Search query: AGGTCTTCAA, Tissues and cell lines

<http://cgap.nci.nih.gov/>

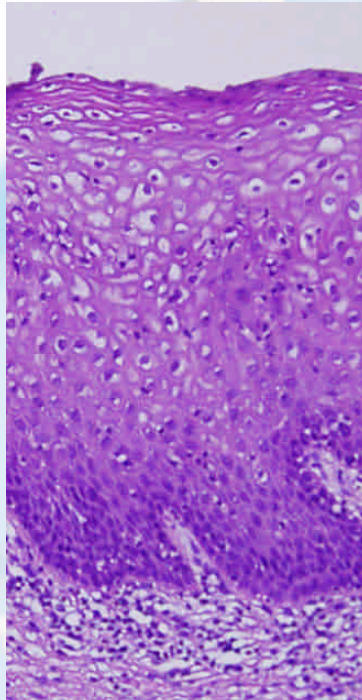


Q: What is the role of TSP-1 in cervical multi-step carcinogenesis?

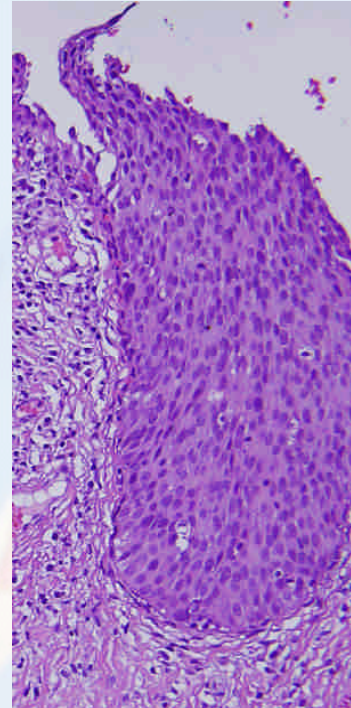
Normal



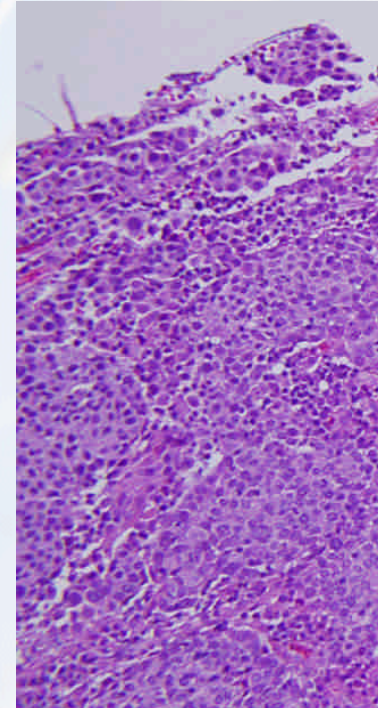
LSIL



HSIL



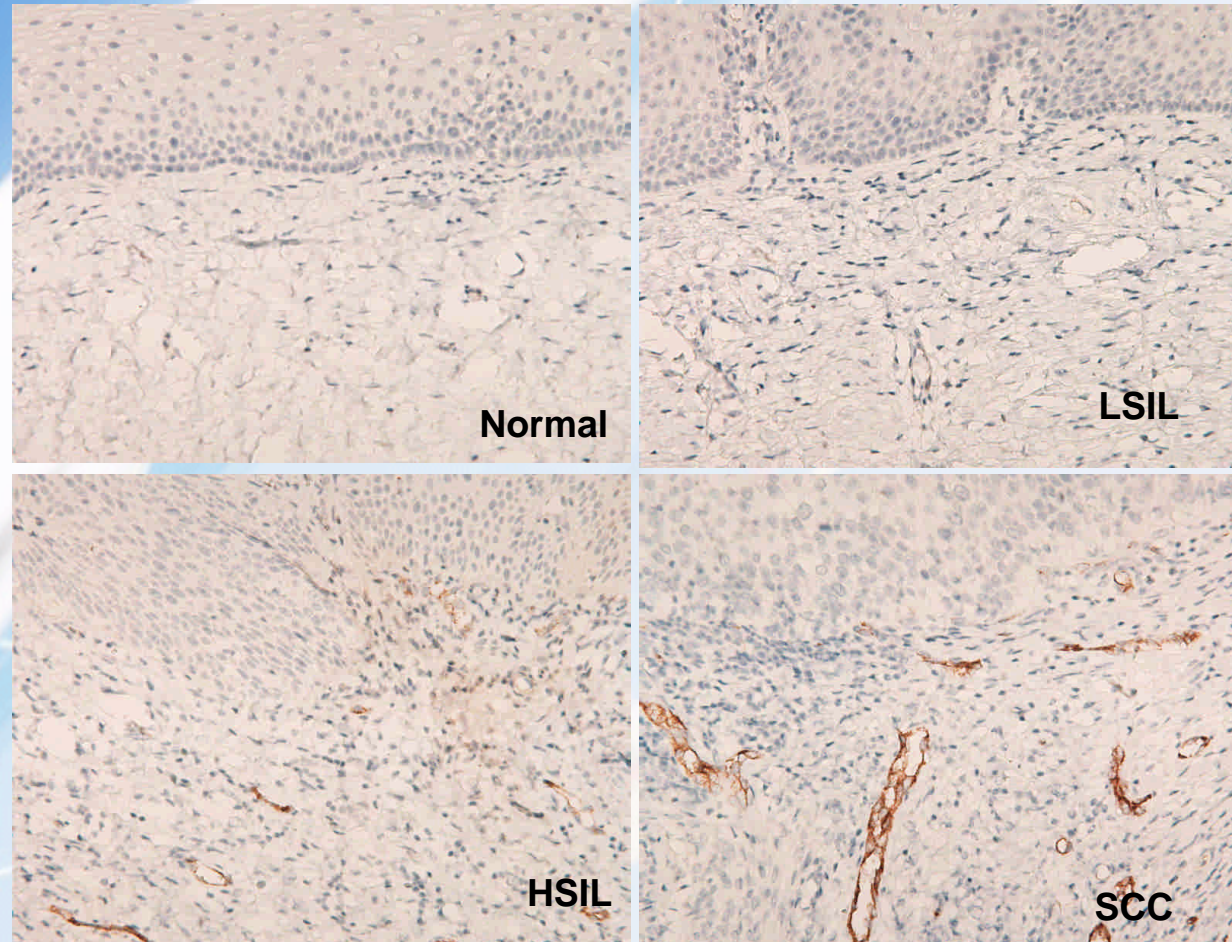
SCC



L(H)SIL: low (high)-grade squamous intraepithelial lesions

Microvessels in different cervical lesions

Fig. 1

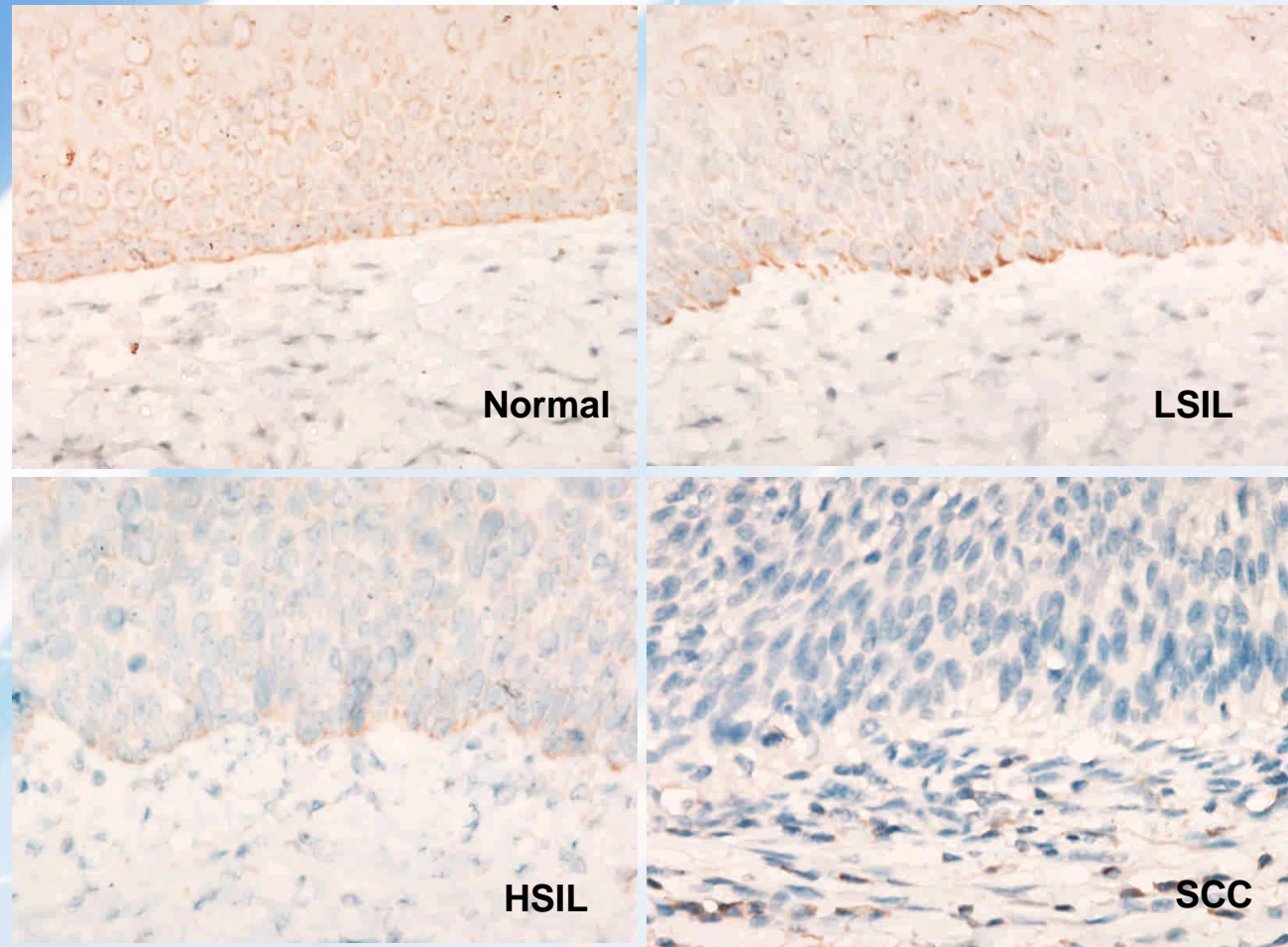


LSIL: low squamous intraepithelial lesion

HSIL: high squamous intraepithelial lesion; SCC: squamous cell carcinoma

TSP-1 expression in different cervical lesions

Fig. 2



LSIL: low squamous intraepithelial lesion

HSIL: high squamous intraepithelial lesion; SCC: squamous cell carcinoma

TSP-1 (TSP-1 fence) down-regulation coincides with the angiogenic switch during cervical carcinogenesis

Table. 1

Cervical lesions	MVD			TSP-1 score	
	No.	Mean \pm SD	p-value [#]	Mean \pm SD	p-value [#]
Normal	48	7.3 \pm 2.9	<0.001	3.8 \pm 0.4	<0.001
LSIL	36	9.9 \pm 3.4		3.8 \pm 0.4	
HSIL	24	17.7 \pm 5.1		1.8 \pm 0.4	
SCC	12	22.8 \pm 8.6		1.5 \pm 0.5	

Results (part I)



- The disruption of “TSP-1 fence” (TSP-1 was mainly localized at the basal epithelial cells) and the switch to angiogenic phenotype occurred during the transition from LSIL into HSIL.
- This concordance suggests that TSP-1 play a role in the regulation of angiogenic switch.
- The onset of angiogenesis is an early event in cervical carcinogenesis due to, in part, the down-regulation of TSP-1 by the **dysplastic epithelium**.

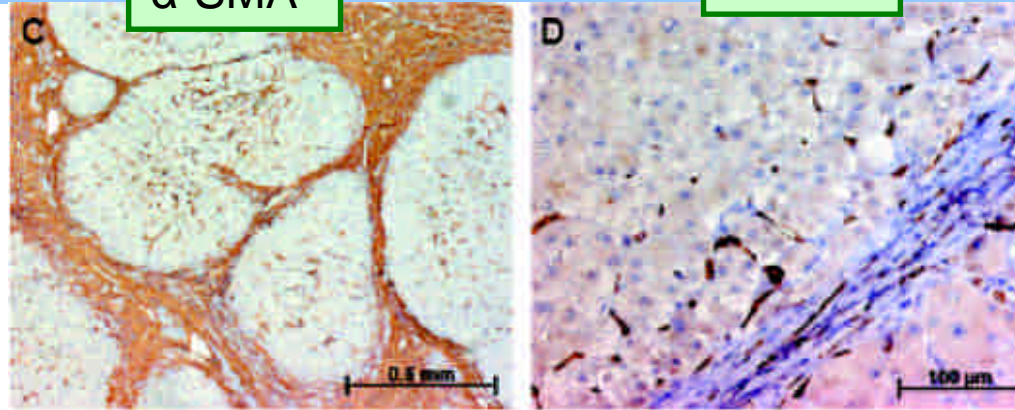
Wu MP, Chou CY et. al. 2004 Cancer J (SCI, IF: 2.028, Oncology 78/127)

Stroma reaction is characterized by stroma marker overexpression

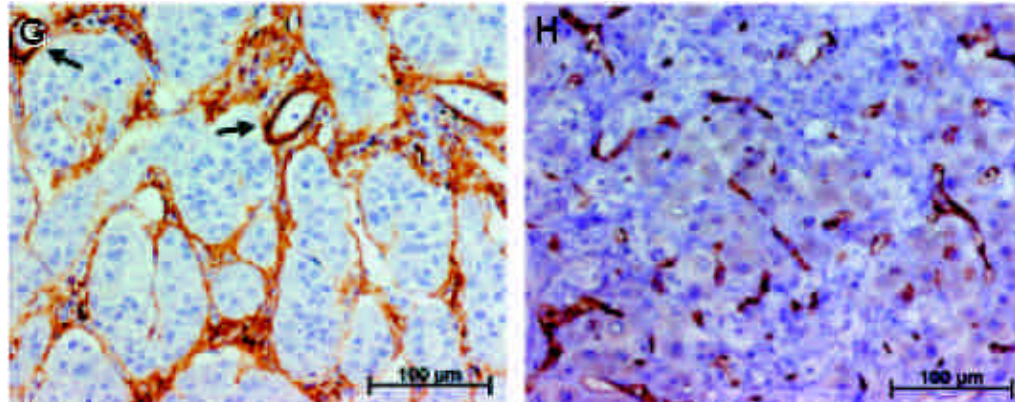
α -SMA

CD34

Liver cirrhosis

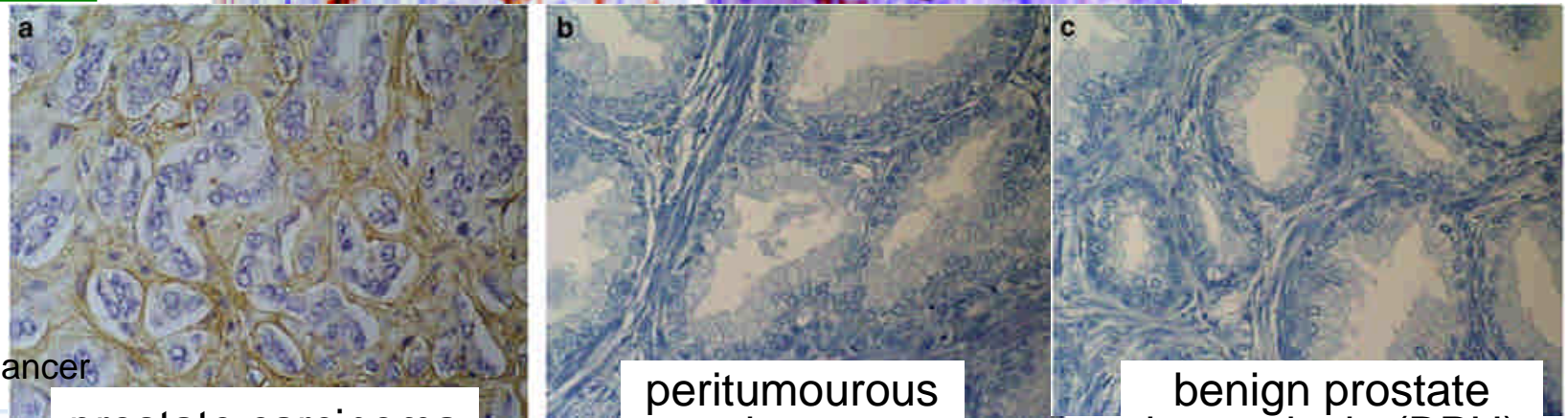


Hepatocellular Ca



Tenascin-C

Prostate



Tomas D et al.
2006 Prostate Cancer
Prostatic Dis

prostate carcinoma

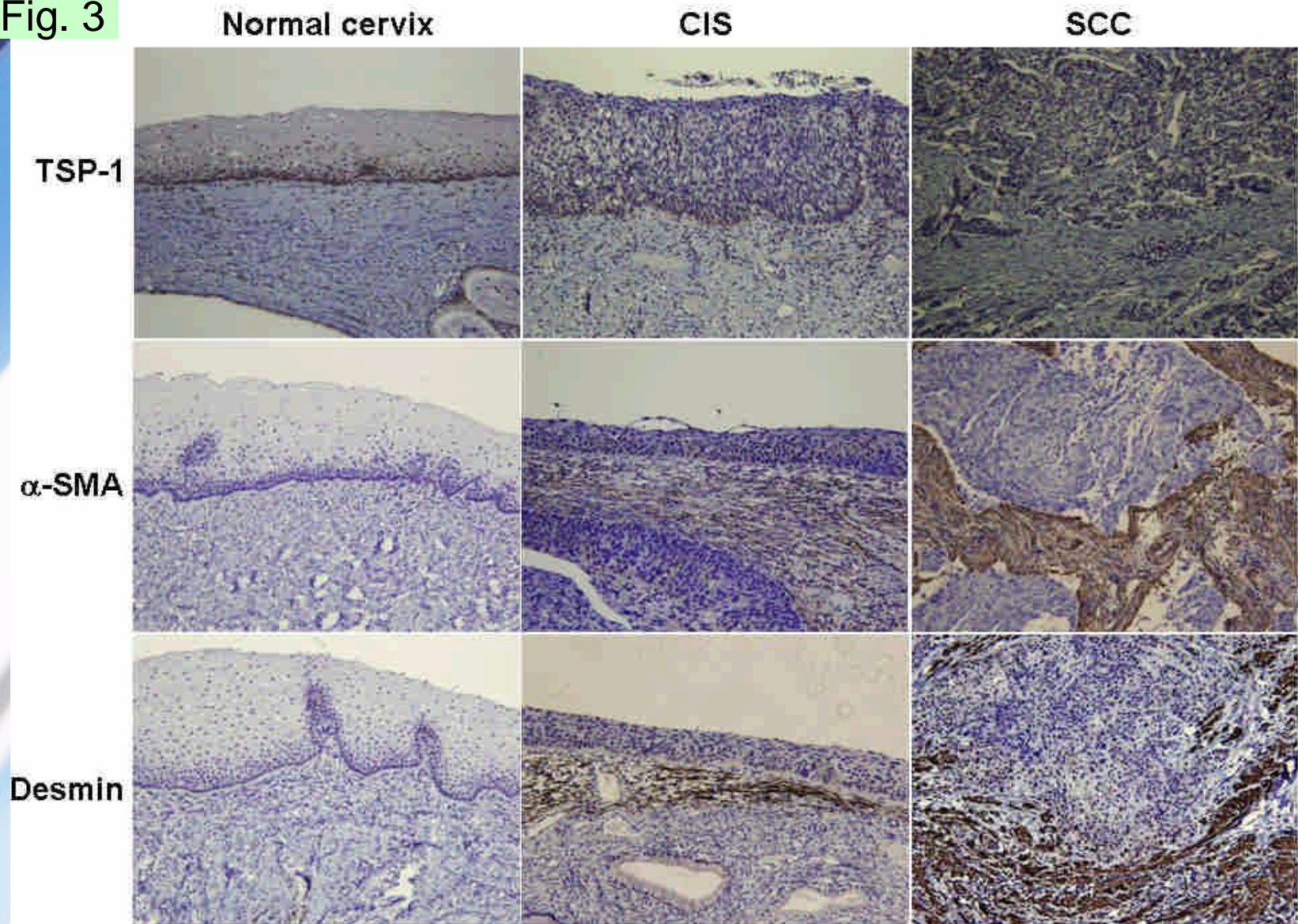
peritumorous
tissue

benign prostate
hyperplasia (BPH)

Desmoulier A et al.
2004 Int J Dev Biol

The expression of TSP-1, and two stroma markers (α -SMA and desmin) during cervical carcinogenesis

Fig. 3



Distribution of two stroma markers, **α -SMA** and **desmin** scores in Normal, CIS or SCC

Lesions	α -SMA			desmin			
	No.	Mean \pm SD	p-value	Lesion	No.	Mean \pm SD	p-value
Normal ^{a,c}	15	0.67 \pm 0.42	<0.001	Normal ^{d,f}	15	0.21 \pm 0.18	<0.001
CIS ^{a,b}	15	2.13 \pm 0.61		CIS ^{d,e}	15	0.57 \pm 0.31	
SCC ^{b,c}	15	2.78 \pm 0.21		SCC ^{e,f}	15	1.78 \pm 0.44	

Table 3. Kruskal-Wallis test with Dunn post hoc comparison test normal cervix (Normal), carcinoma in situ (CIS) or invasive squamous cell carcinoma (SCC); a: 0.002, b: 0.006, c: < 0.001; d; 0.019, e: <0.001, f: <0.001

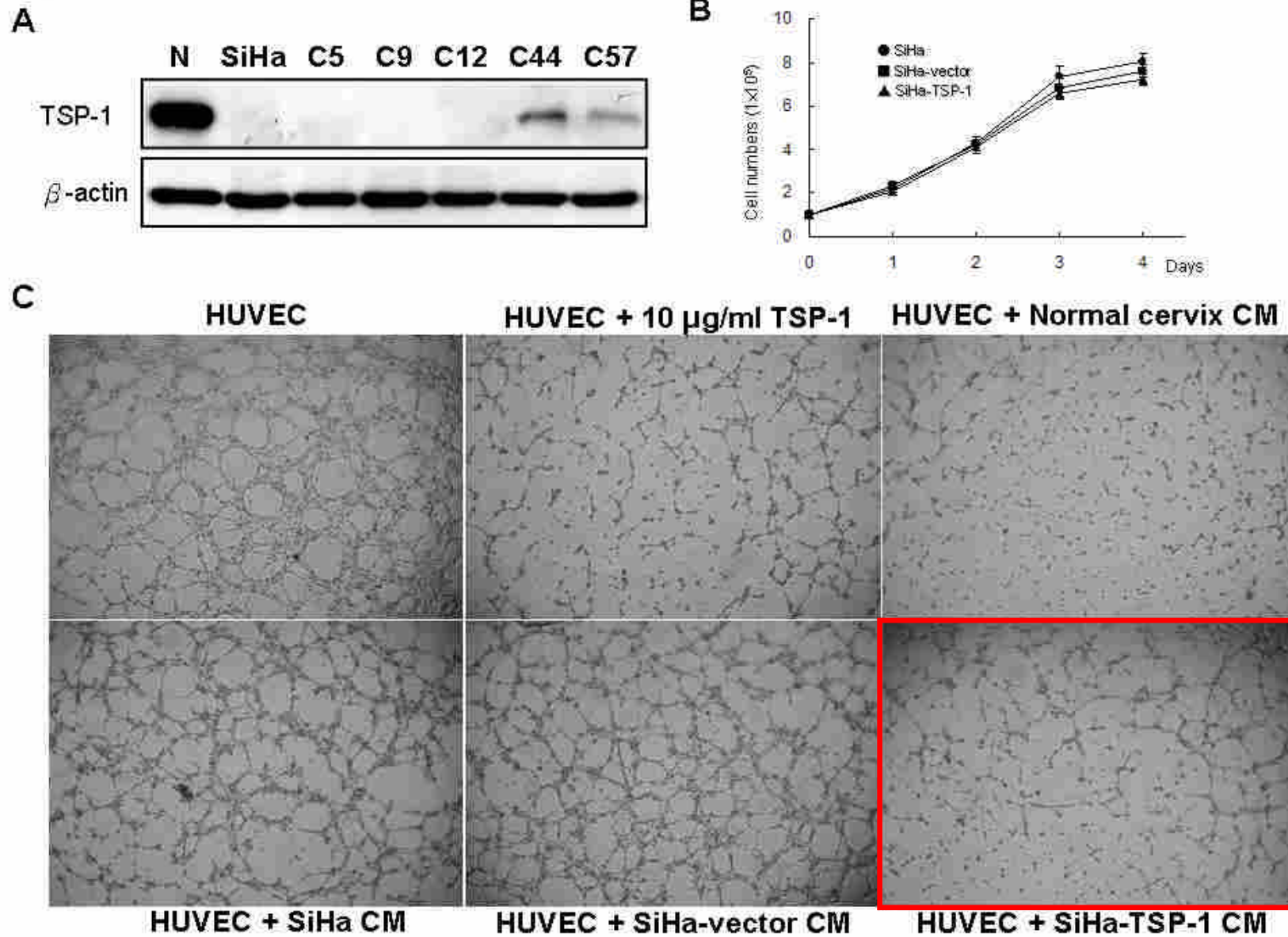
Results (i)

- The disappearance of TSP-1 is coincided with the emergence of stroma markers during cervical carcinogenesis
 - in surgical specimens
- It suggests that TSP-1 plays a role in stroma reaction.



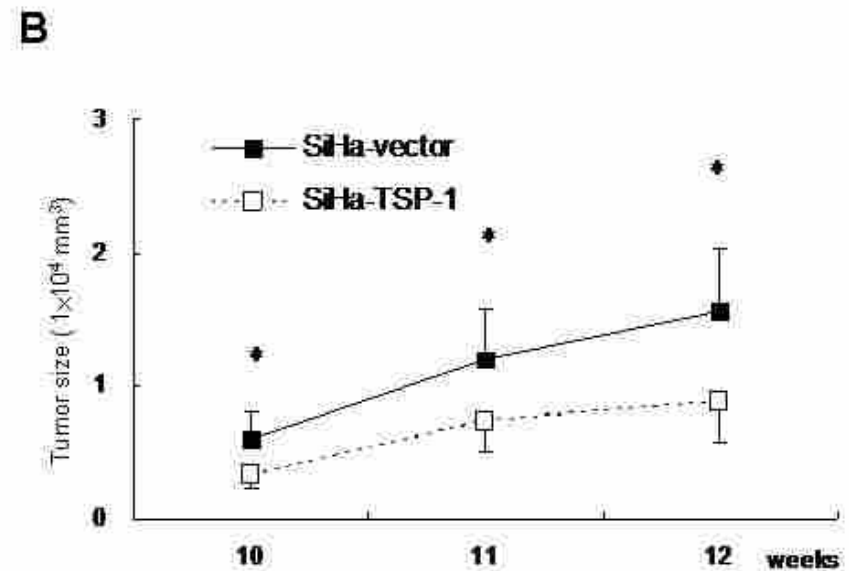
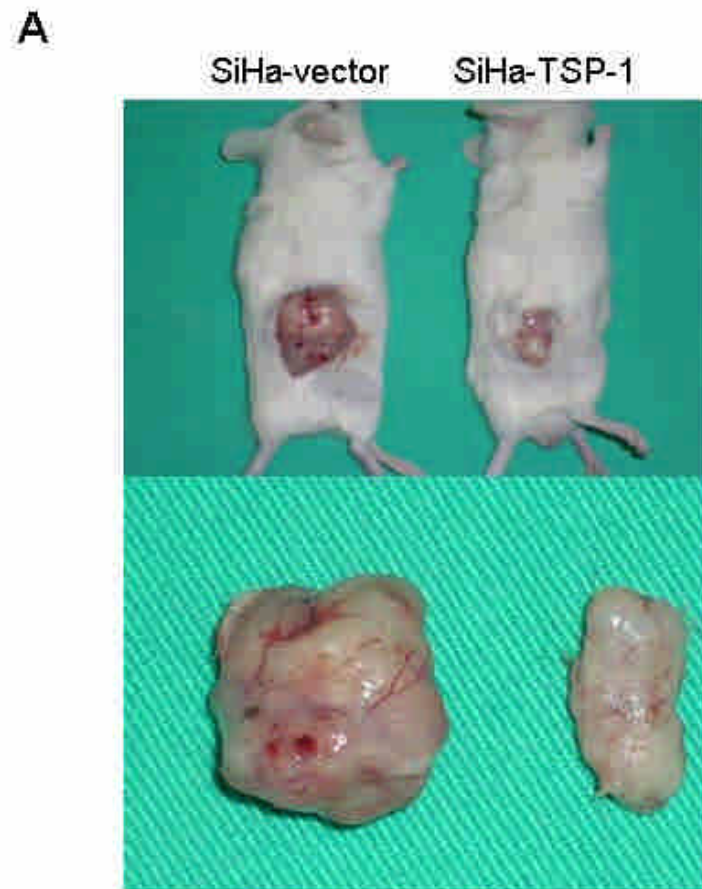
The characterization of TSP-1-overexpressing clones

Fig. 4



TSP-1 inhibits tumor development in SCID xenograft model

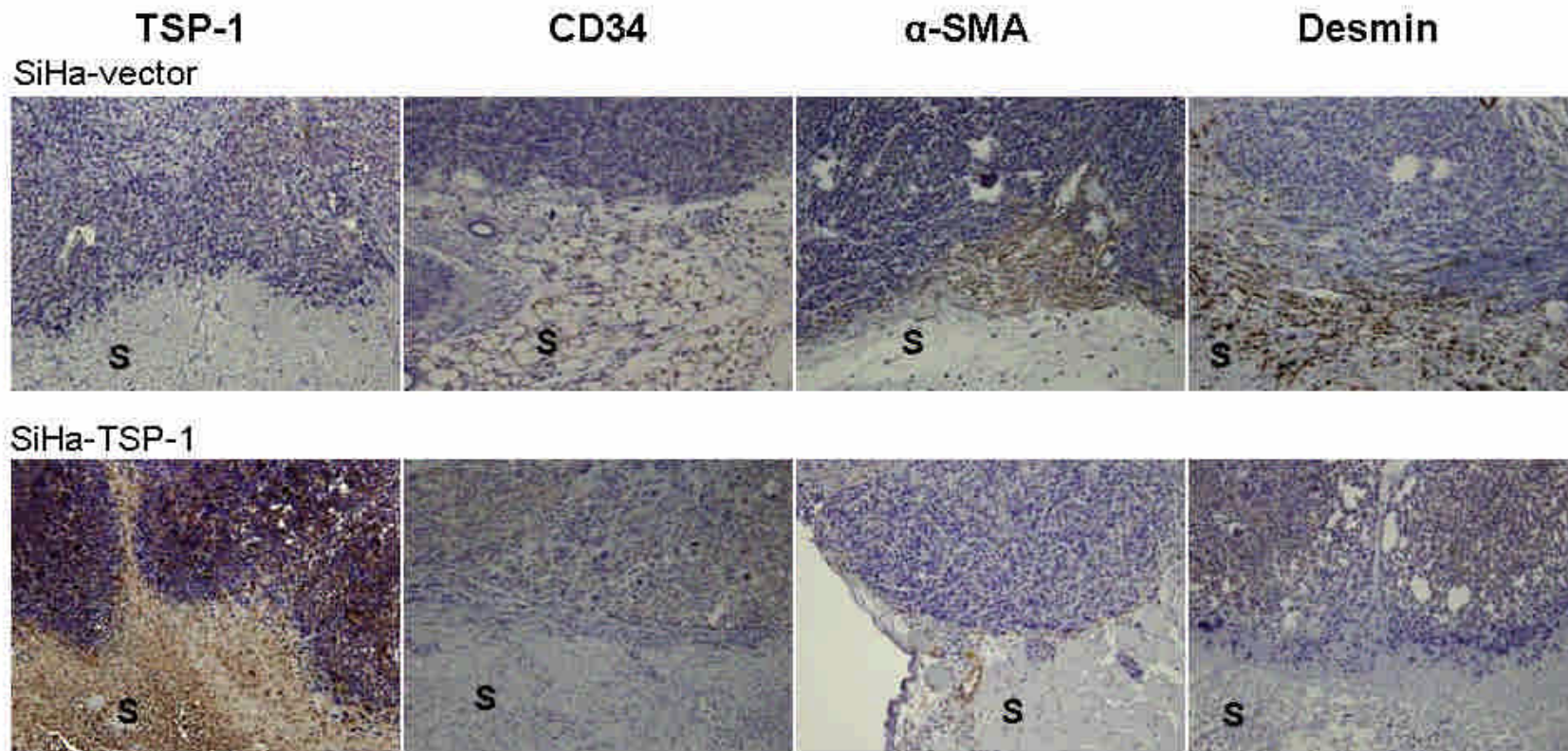
Fig. 5



TSP-1 inhibits angiogenesis, and SCC-induced stromal reaction in vivo

Fig. 5

C

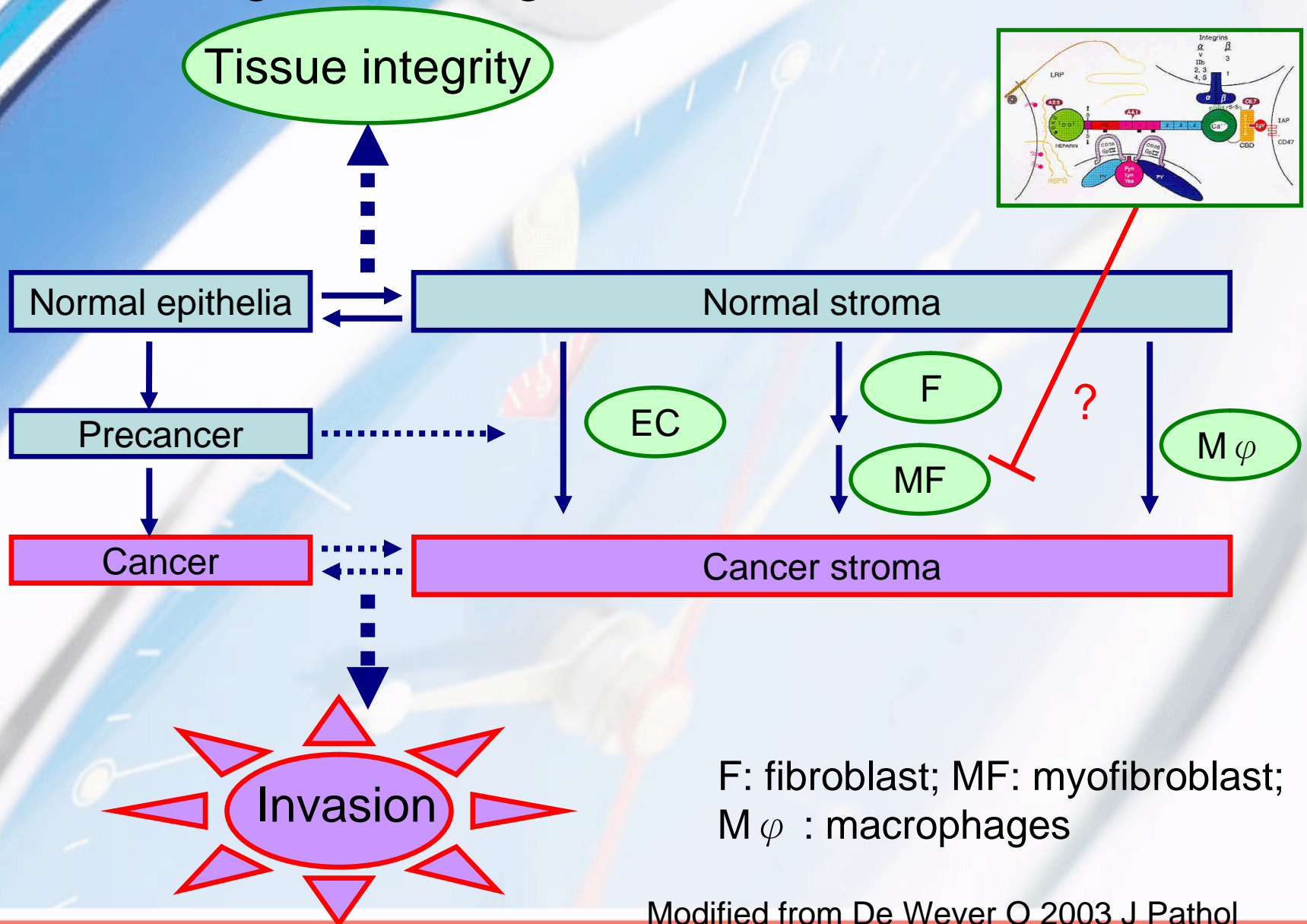


Results (ii)

- TSP-1 inhibits tumor development, and SCC-induced stromal reaction *in vivo*
 - reduced **tumor growth** in SCID mice,
 - decreased **tumor vascularization**
(CD34, microvessel density)
 - lower level of **stroma markers**
(α -SMA and desmin expression)



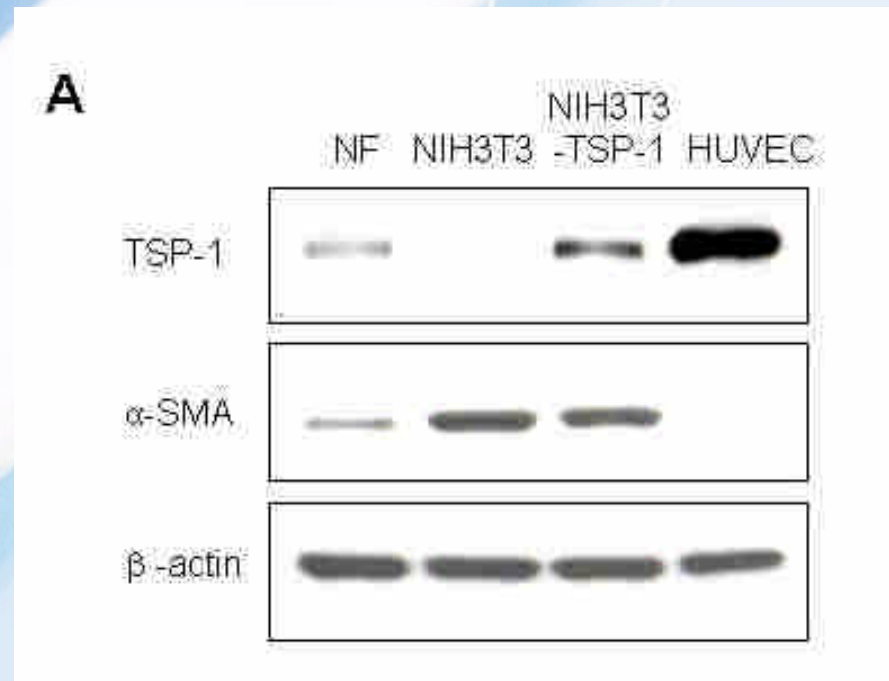
Q: Can TSP-1 inhibit stroma reaction through inhibiting fibroblast activation?



Modified from De Wever O 2003 J Pathol

To establish TSP-1-overexpressing-NIH3T3

Fig. 6



- NF: normal human fibroblast
- NIH3T3: modestly α -SMA- expressing fibroblast
- NIH3T3-TSP-1: TSP-1-overexpressing NIH3T3

Stroma reaction is characterized by the fibroblast activation

- Fibroblasts

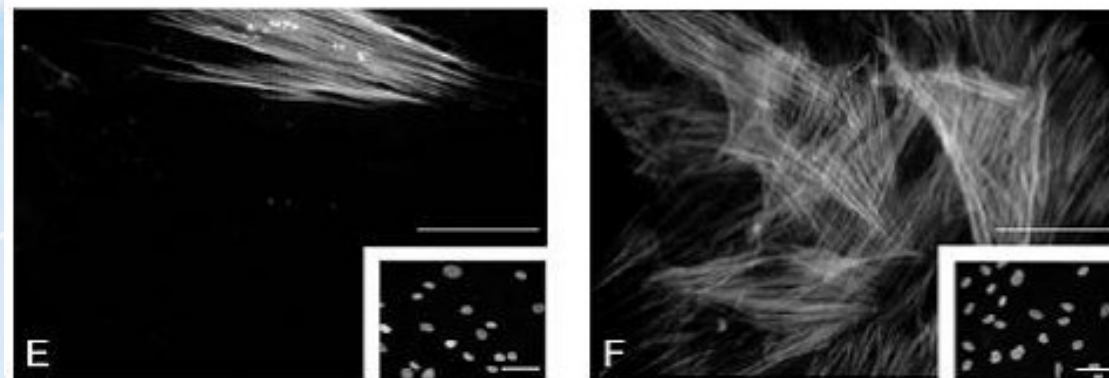
- are recruited and converted into α -SMA-positive fibroblasts,
- i.e., **myofibroblasts** or **activated fibroblasts**, during stroma reaction (Kunz-Schughart LA and Knuechel R Histol Histopathol)
- Produce noncellular scaffolds known as ECM proteins in response to several extracellular stimuli



fibroblast

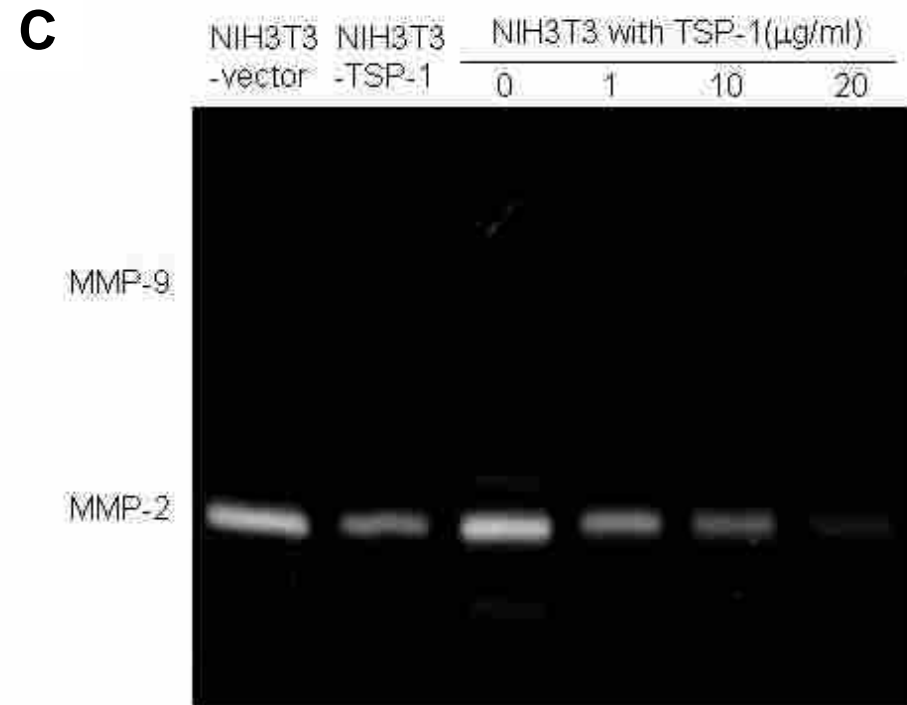
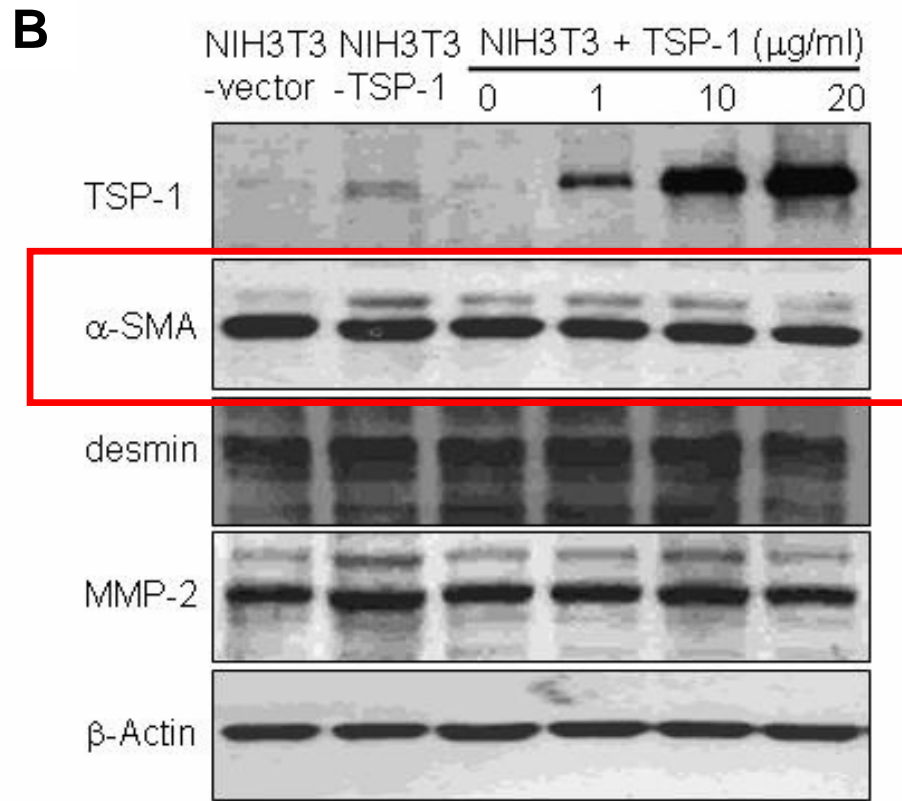
myofibroblast

α -SMA



TSP-1 does not downregulate α -SMA and desmin expression, but inhibits MMP-2 activity

Fig. 6

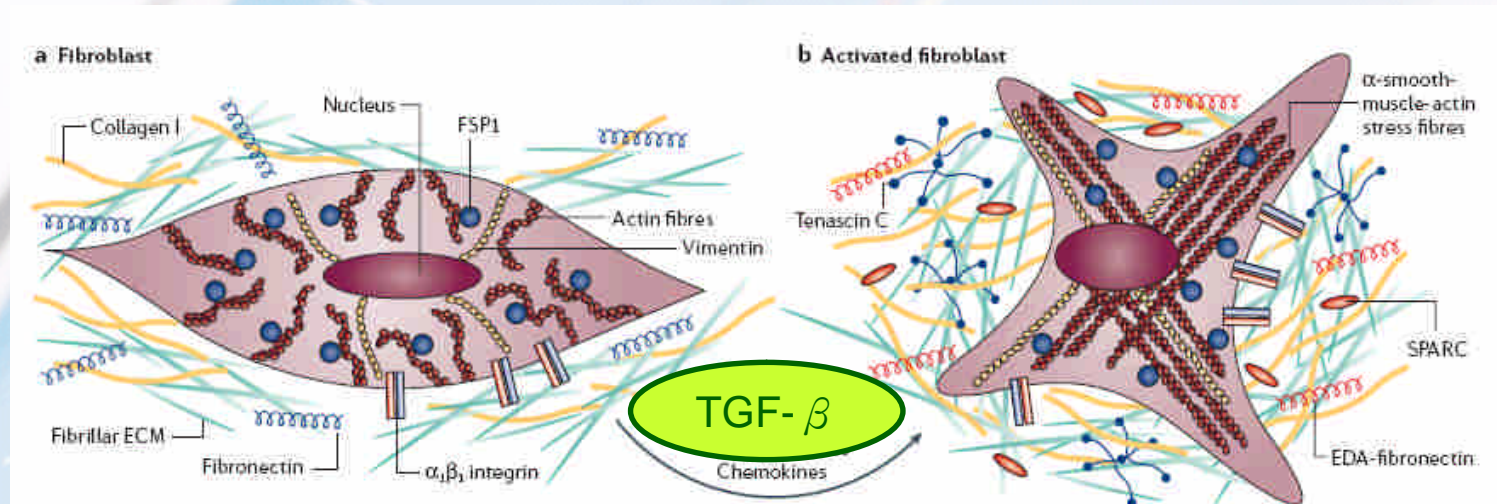


TGF- β -induced fibroblast transdifferentiation



- Although NIH-3T3s express some α -SMA,
 - these cells are regularly used to study myofibroblastic differentiation by various stimuli
 - such as P311, **TGF- β** , nerve growth factor, tumor-derived ECMs and more.

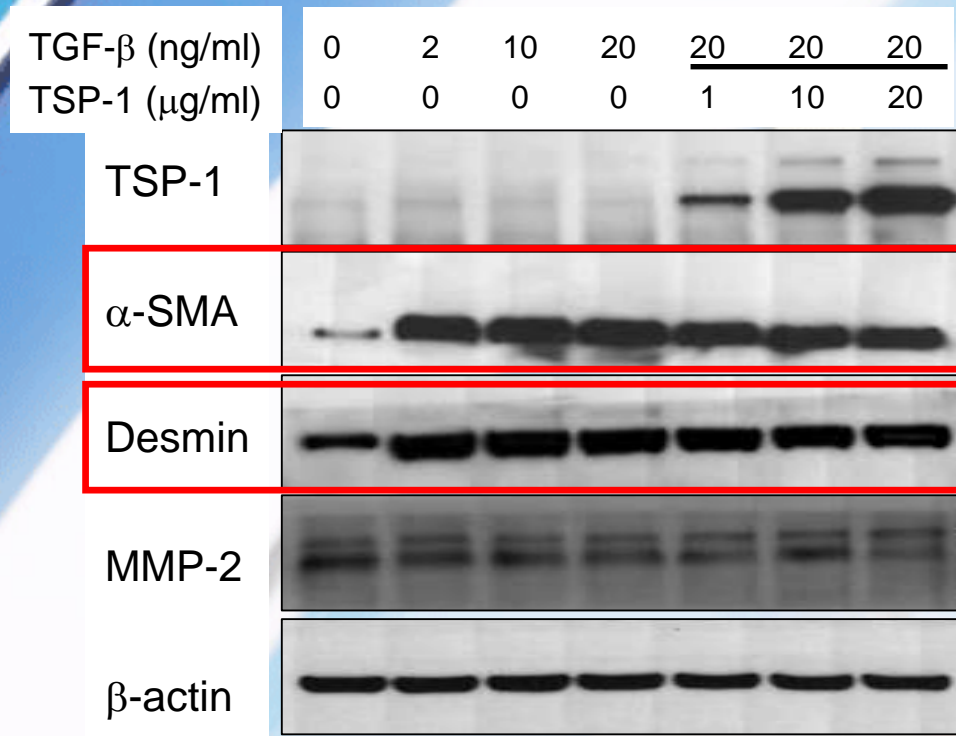
(Comments from the reviewers)



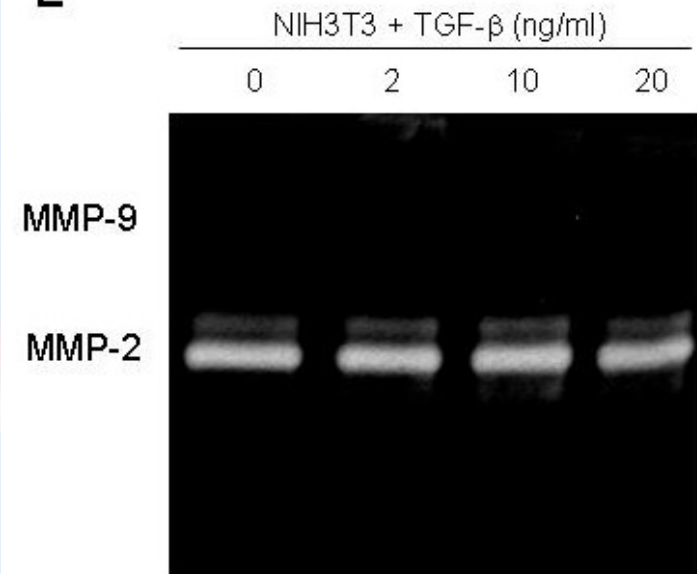
TGF- β upregulates α -SMA and desmin expression, but does not change MMP-2 activity

Fig. 6

D



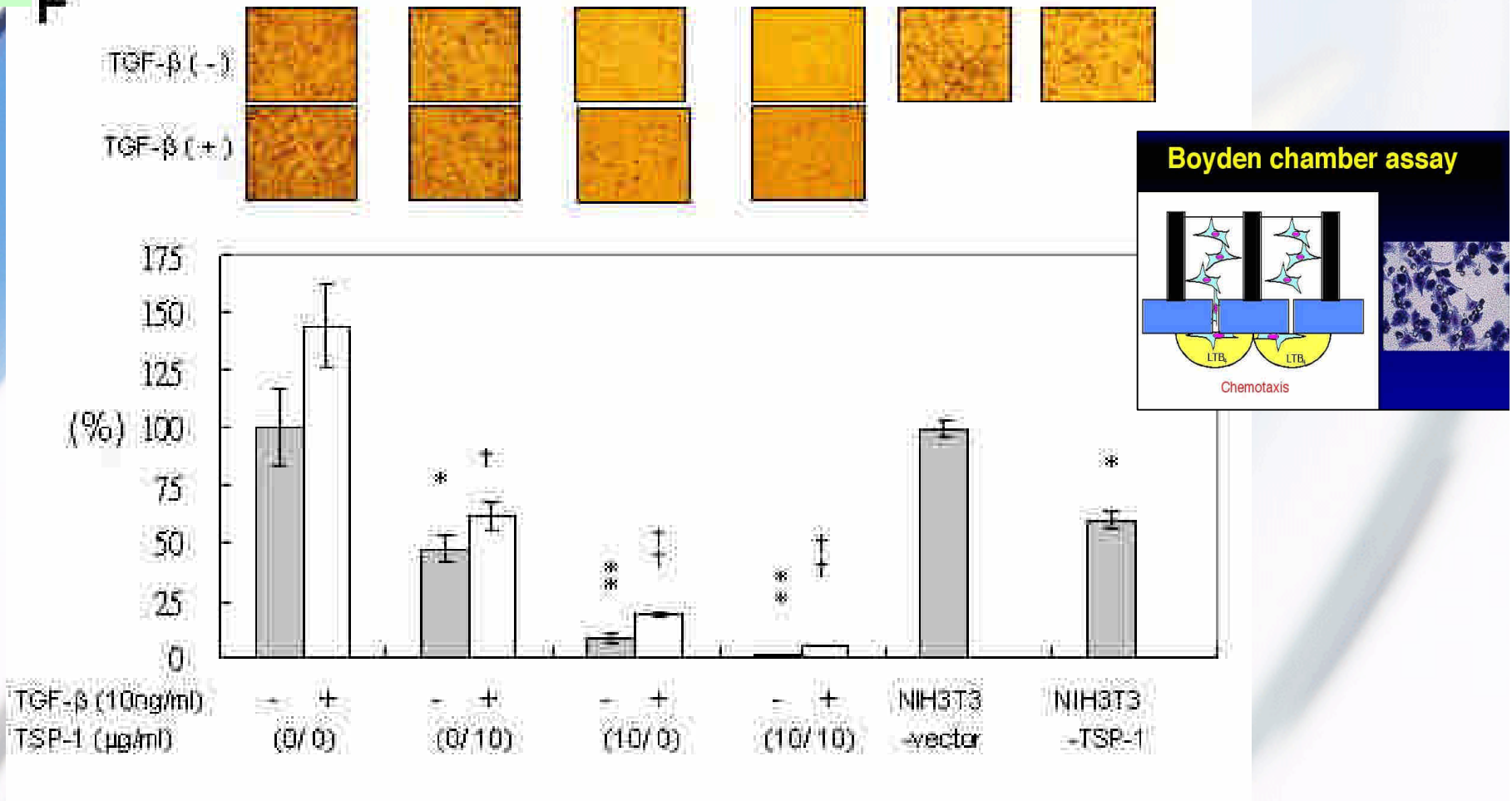
E



TSP-1 inhibits the migration of untreated and TGF- β -treated NIH3T3 cells

fig. 

Fig. 6 **F**



Results (iii)



- TGF- β treatment on NIH3T3
 - upregulates α -SMA and desmin expression
 - does not downregulate MMP-2 in protein level
 - does not downregulate MMP-2 activity (zymography)
 - increases migration ability
- Elevated levels of TSP-1 on NIH3T3
 - does not change α -SMA and desmin expression
 - does not downregulate MMP-2, MMP-9 at protein level
 - inhibits MMP-2 activity (zymography)
 - inhibits migration ability
 - (dose-dependent pattern)
- TSP-1 inhibits the migration of untreated and TGF- β treated NIH3T3 cells

The effect of TSP-1 & TGF- β on fibroblast activation and trans-differentiation

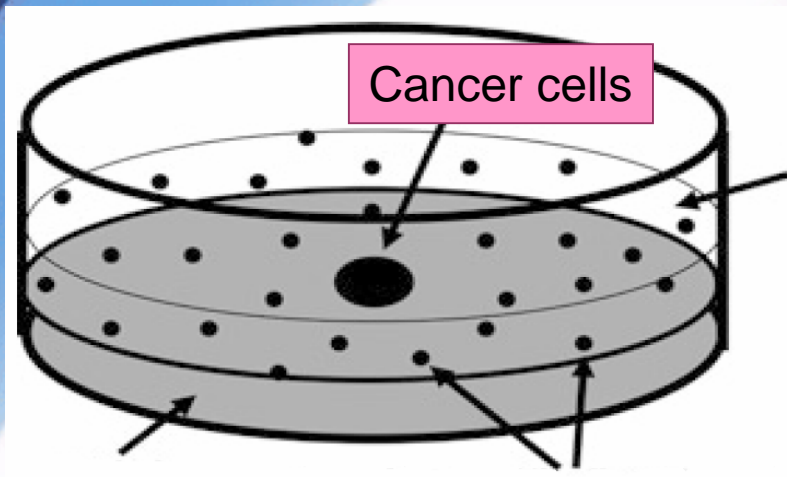
	TSP-1	TGF- β
α -SMA	No change	increase
Desmin	No change	increase
MMP-2 (western)	No change	No change
MMP-2 (zymography)	decrease	No change
Migration	decrease	increase
Invasion (matrigel)	decrease	increase

Myofibroblasts and tumor progression

- Myofibroblasts are present in the stroma of many malignant tumors
 - are frequently localized at the front of invasion
(De Wever O and Mareel M 2003 J Pathol)
- Create a context to promote tumor progression
(Barcellos-Hoff MH & Ravani SA 2000 Cancer Res).
- Closely linked to tumor progression, lymph node and distant organ metastasis (Hasebe T, et al. 2000 Am J Pathol)
- **Have a migratory and invasive capacity similar to cancer cells**
 - associate with cancer cells by transmitting reciprocal signals (Ishii G, et al. 2005 Int J Cancer).

Q: Can TSP-1 block the invasive ability of the myofibroblasts?

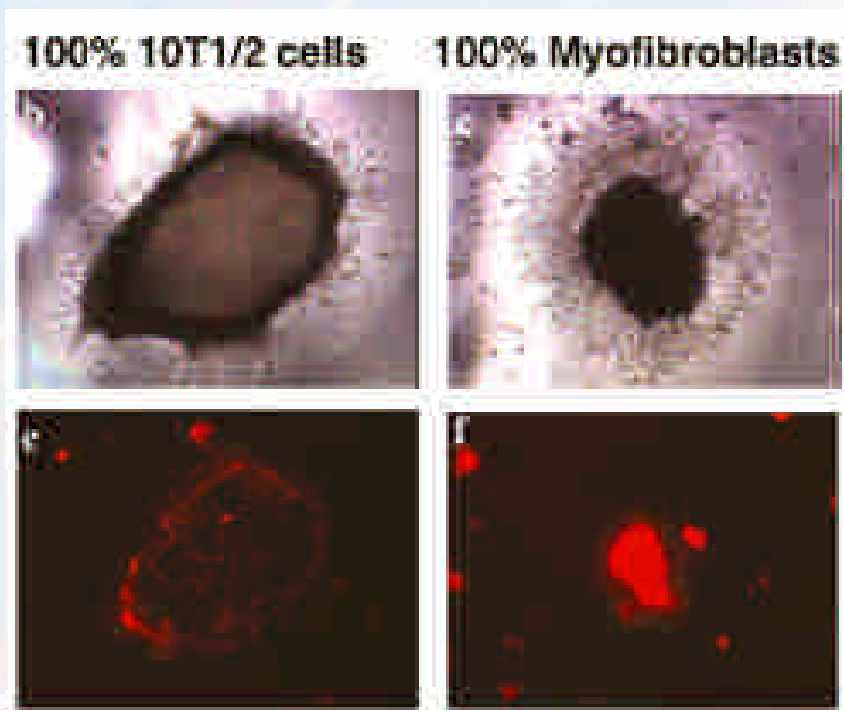
Matrigel fibroblast invasion assay



Matrigel

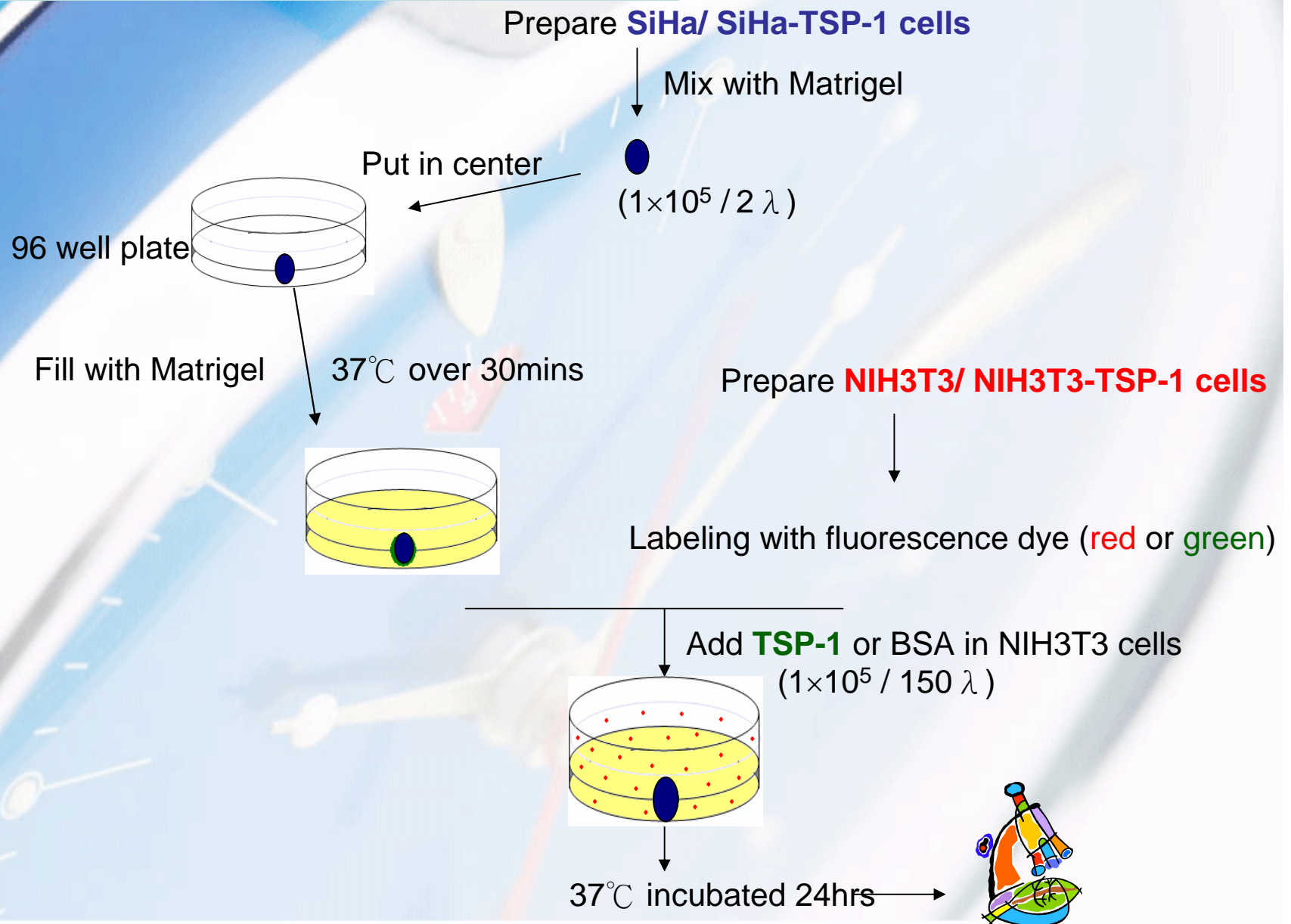
(myo)fibroblasts

10T1/2 mesenchymal cells



Modified from Walter-Yohring J, et al.
2003 Cancer Chemother Pharmacol

Myofibroblast invasion assay with Matrigel multicellular coculture system

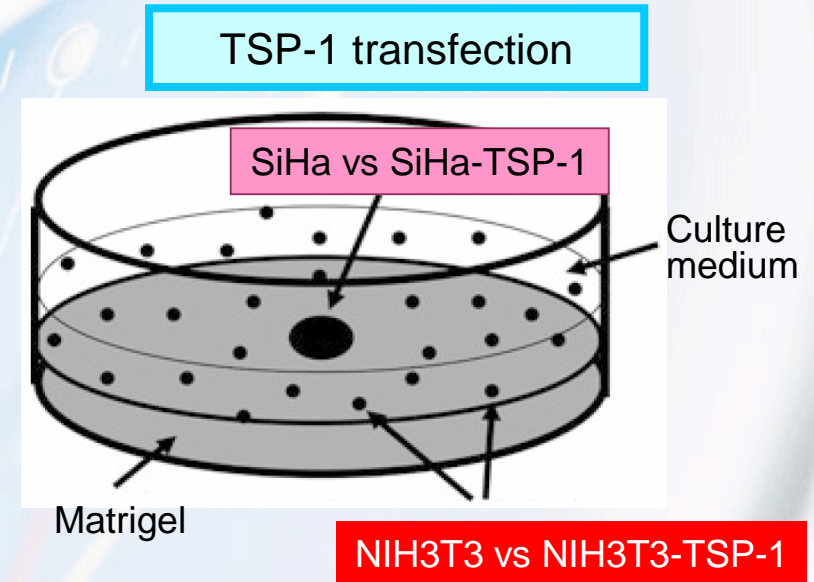


Study design: The inhibitory effect of TSP-1 on the invasive ability of myofibroblasts

Matrigel fibroblast invasion assay

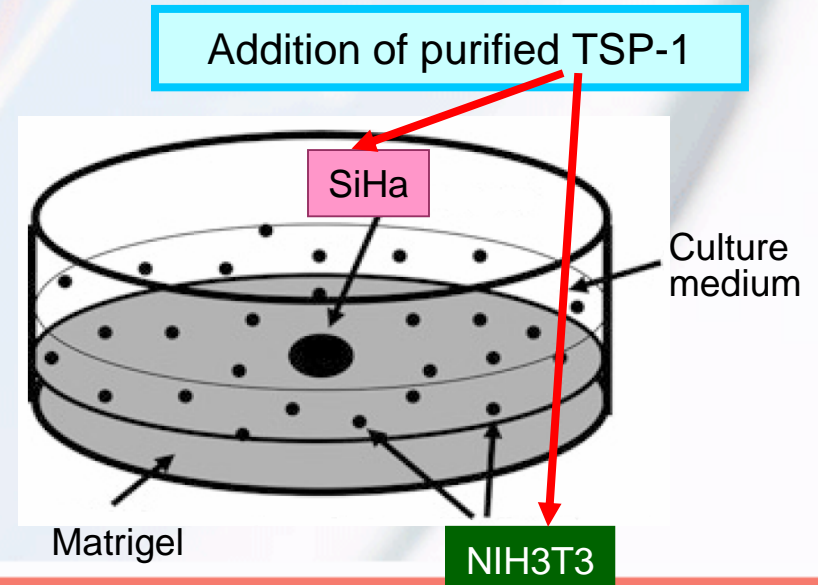
(a) TSP-1 transfection

	SiHa	SiHa-TSP-1
NIH3T3		
NIH3T3-TSP-1		



(b) Addition of purified TSP-1

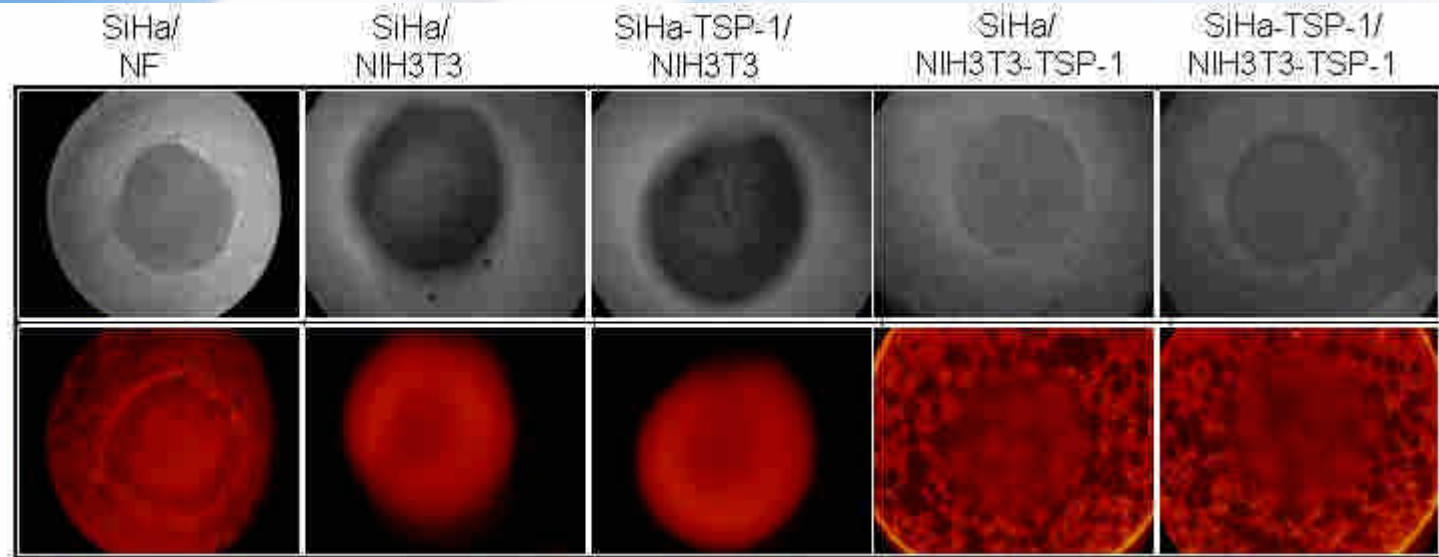
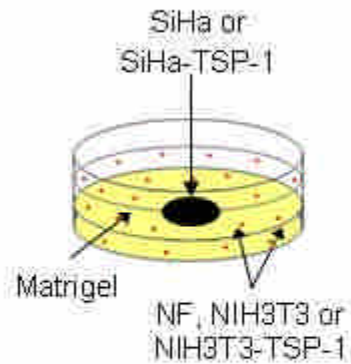
	TSP-1			
SiHa	-	-	+	+
NIH-3T3	-	+	-	+



TSP-1 (by transfection) exerted its anti-invasive ability in activated fibroblast, but not tumor cells

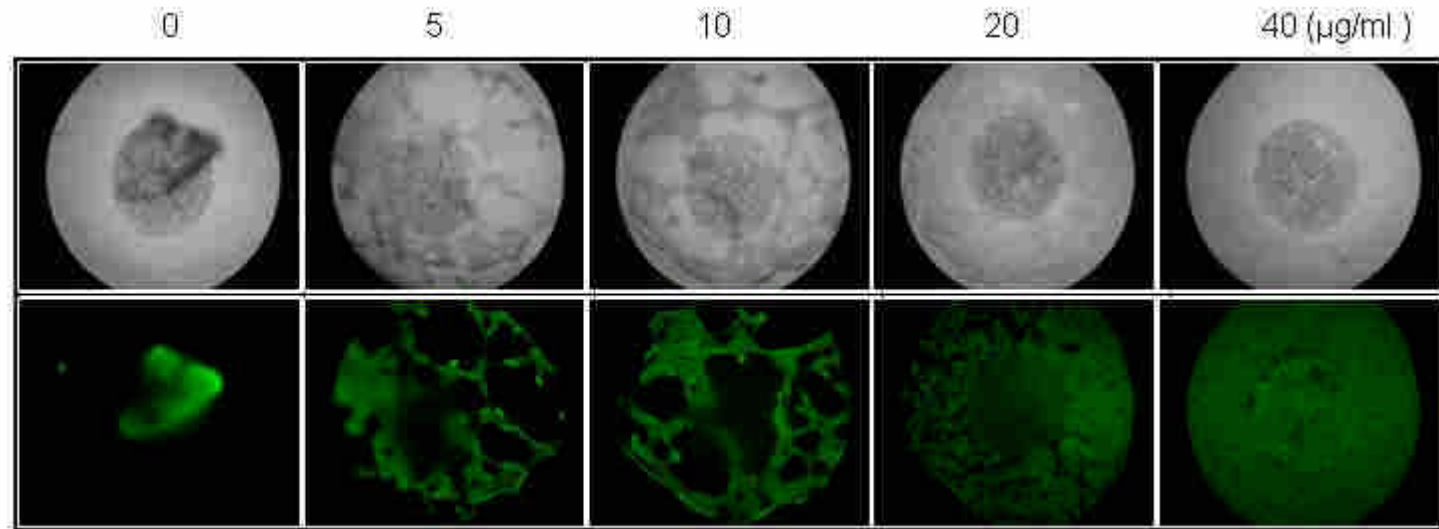
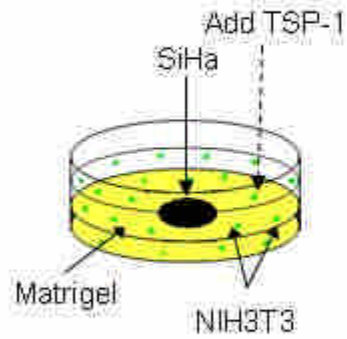
Fig. 7

A



TSP-1 (by addition) exerted its anti-invasive ability in untreated and TGF- β -treated activated fibroblasts


Fig. 7
C



Results (iv)



- Elevated levels of TSP-1 on fibroblasts potently inhibits myofibroblasts from invading tumor cell cluster
 - by plasmid transfection
 - by direct addition of purified TSP-1
 - Regardless the presence of TGF- β
 - demand a higher dose for a complete inhibition
- TSP-1-mediated inhibition of the invasive ability via (myo)fibroblasts, but not cancer cells

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	Journal Name	Art. No.	CE Code

Carcinogenesis vol.0 no.0 pp.1–9, 2008

doi:10.1093/carcin/bgn077

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A novel role of thrombospondin-1 in cervical carcinogenesis: inhibit stroma reaction by inhibiting activated fibroblasts from invading cancer

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Thrombospondin (TSP)-1, a potent angiogenesis inhibitor, has been shown to exert different biological functions on various cell types. Here, we investigate the role of TSP-1 in tumor–stroma reaction, which is mainly characterized by fibroblast activation to create a permissive microenvironment for tumor progression. Immunohistochemistry examinations in the human surgical specimens have shown that a downregulation of TSP-1 during the progression of cervical carcinogenesis was accompanied by an emergence in the upregulation of stroma markers, α -smooth muscle actin (α -SMA) and desmin. Transfection of SiHa cervical cancer cells with a plasmid expressing the TSP-1 protein exhibited antiangiogenic activity *in vitro* and resulted in reduced tumor growth in severe combined immunodeficiency (SCID) mice, which was accompanied by a decrease in tumor vascularization and lower expressions of α -SMA and desmin than those in the vector controls. Transfection with TSP-1 and purified TSP-1 added to NIH3T3 cells did not alter the protein levels of α -SMA and desmin but significantly inhibited matrix metalloproteinase-2 activity. Transforming growth factor- β (TGF- β), a major factor in the activation of fibroblasts, increased α -SMA and desmin expression and the ability of cell migration and invasion in NIH3T3 cells. The increased migration ability and the invasive ability into tumor

Discussion

WASHINGTON UNIVERSITY NOBEL LAUREATES



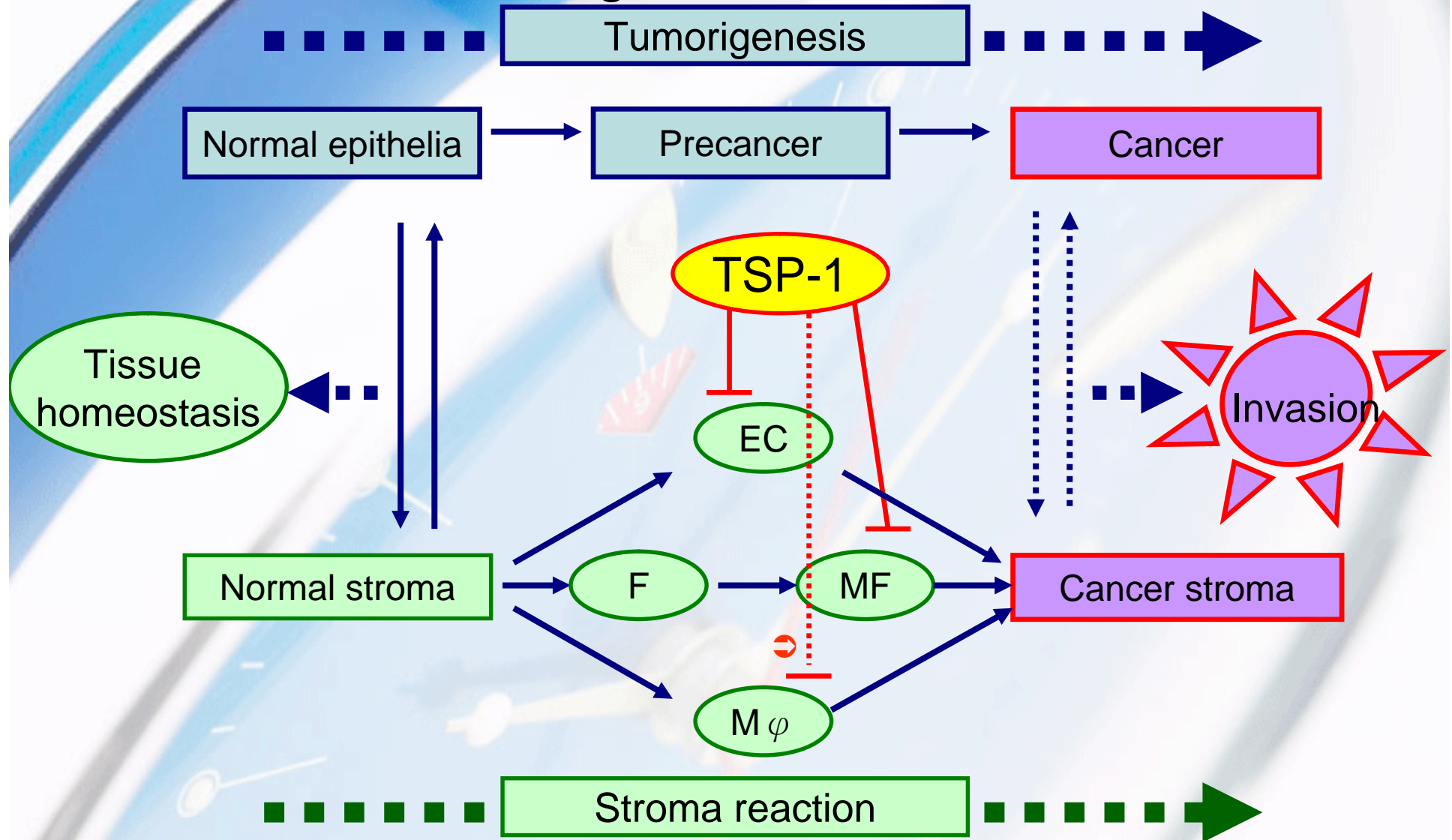
(2004.05 - 2005.05 國科會千里馬計劃)

Sea shell Lobby, School of Medicine, Washington University, St. Louis, MO

Our evidence shows that TSP-1 has the potential to inhibit stroma reaction

- (i) The concordance of the disappearance of TSP-1 and the emergence of stroma markers
 - in surgical specimens
- (ii) TSP-1-overexpressing-SCC (vs vector control)
 - reduced **tumor growth** in SCID mice
 - decreased tumor **vascularization**
 - lower level of **stroma markers** (α -SMA and desmin)
- (iii) A gain-of-TSP-1-function in fibroblasts inhibits myofibroblasts from invading tumor cell clusters
 - TSP-1 transfection
 - addition of purified TSP-1.

Summary: TSP-1 inhibits cancer progression via inhibiting stroma reaction



F: fibroblast; MF: myofibroblast; M_{φ} : macrophages

The differences between TSP-1 & SPARC on the trans-differentiation of fibroblasts

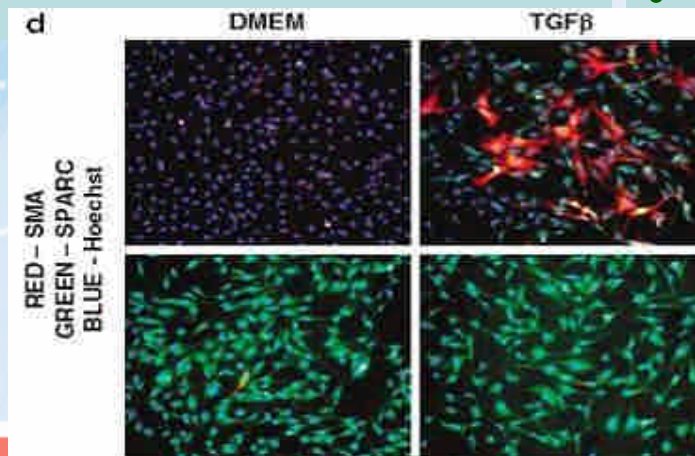
TSP-1

- Matricellular protein
- Anti-angiogenesis
- Interfering ECM
- **little effect on the α -SMA expression**
- **Inhibits myofibroblast migration and invasion**

SPARC*



- Matricellular protein
- Anti-angiogenesis
- Interfering ECM
- **prevents fibroblast transdifferentiation**
 - **downregulates α -SMA**
- **enhances normal fibroblast migration ability**
- **increases unresponsive tumor stroma formation**





*SPARC: secreted protein, acidic and rich in cysteine

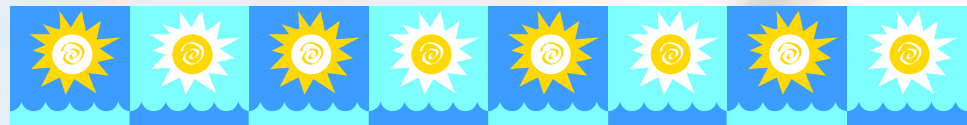
Chlenski A, et al. 2007 Oncogene

TSP-1 involves almost all of the processes of stromagenesis

- **Stromal cells can contribute oncogenic signals**

(Tlsty TD, et al. 2001)

- **exposure to carcinogens, e.g. ras** (Kalas W, et al. 2005 Cancer Res) 
- **manipulation of matrix metallo-proteinases (MMPs)** (Rodriguez-Manzaneque JC, et al. 2001 PNAS) 
- **The recruitment of inflammatory cells to the stroma** (Doyen V 2003, et al. J Exp Med; Vallejo AN, et al. 2000 J Immunol)



The dualistic roles of TSP-1 in tumor progression

(i) The diverse biologic roles

- Interacts with multiple extracellular macromolecules
- and cell surface receptors, exerting a wide range of functions
(Chen et al., 2000 Matrix Biol; de Fraipont et al., 2001 Trend Mol Med).

(ii) The exposure to high stromal TSP-1 may induce increased expression of angiogenesis activators

- override the anti-angiogenic effects of TSP-1 (Fontana et al., 2005 Int J Cancer).

(iii) Variable roles in the regulation of stroma reaction

- Antiangiogenic and proinvasive (Taraboletti G 2000 FASEB J; Wang TN 1996 J Surg Res)

(iv) Some limitations still remain among the various models, e.g. MVD (Hatky L, et al. 2002 J Nat Cancer Inst).

Myofibroblasts in host stroma stimulate cancer invasion: Myofibroblasts themselves are invasive



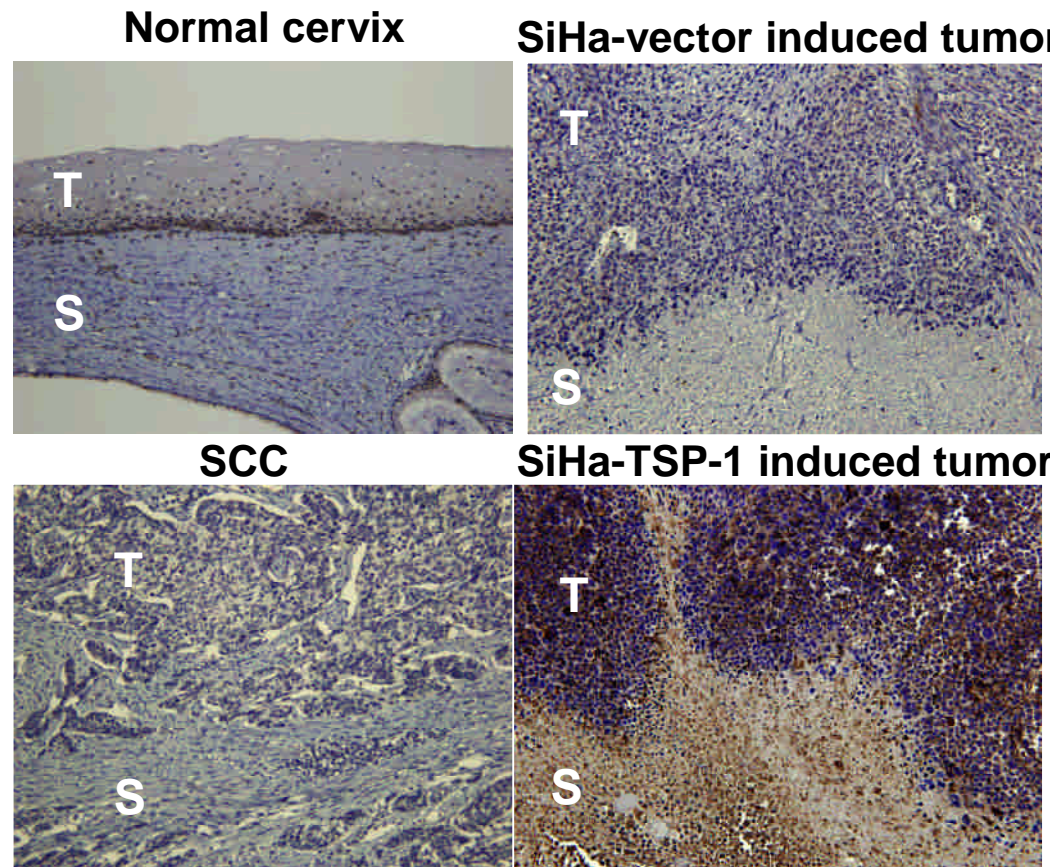
- Stromal cells isolated from surgical colon cancer fragments yielded myofibroblasts,
 - **c.f.** stromal cells from normal mucosa at some distance from the tumor yield normal mucosa (De Wever O *et al.* 2004 FASEB J).
- Present at the front of stroma invasion in many malignant tumors (De Wever O and Mareel M 2003 J Pathol).
- Positively correlated with poor prognosis (Seemayer TA *et al.* 1979 Am J Surg Pathol).
- Myofibroblasts invasion may proceed angiogenic invasion (Fukumura D *et al.* 1998 Cell; Olasso E *et al.* 2003 Hepatology).

Limitation of this study



1. ✓ TSP-1 expression in both the stroma and tumor areas of SCID mouse xenograft vs basal epithelia of normal human cervix

(T/ S: tumor/ stroma)



Possibilities:

- Species differences?
 - Reagent specificity?
 - Study model (heterotopic vs orthotopic ?)
 - Host compensatory effect?
-
- The possibilities of other angiogenic regulators might modulate stroma reaction

Conclusion

The present study expands our understanding of the roles of TSP-1 in tumorigenesis

- The spatial and temporal relationship between TSP-1 expression and MVD was confirmed in human cervical cancer
 - TSP-1 acts as a fence to inhibit angiogenesis that occur during cervical carcinogenesis (Wu MP, Chou CY, et al 2004 Cancer J)
- The inhibitory effects of TSP-1 in stroma reaction extends far beyond the initial observation of its anti-angiogenic role.
 - TSP-1 inhibits stroma reaction by inhibiting activated fibroblasts from invading cancer
(Wu MP, Wu LW, Chou CY, et al. 2008 Carcinogenesis)

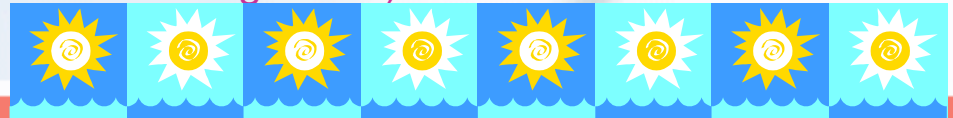


Fig. 8

The effects of TSP-1 on tumor angiogenesis and stroma reaction during cervical carcinogenesis

(Human clinical cervical lesions)

TSP-1 disappearance coincides with angiogenic switch

TSP-1 inversely correlates with stroma marker expression

(SCID mice xenograft model)

TSP-1 decreases microvessel density (MVD) (*in vivo*)

TSP-1 decreases stroma marker expression (*in vivo*)

TSP-1 inhibits tumor growth in SCID xenograft

decreases EC migration, proliferation & induces apoptosis

TSP-1 inhibits migration & invasive ability of myofibroblasts

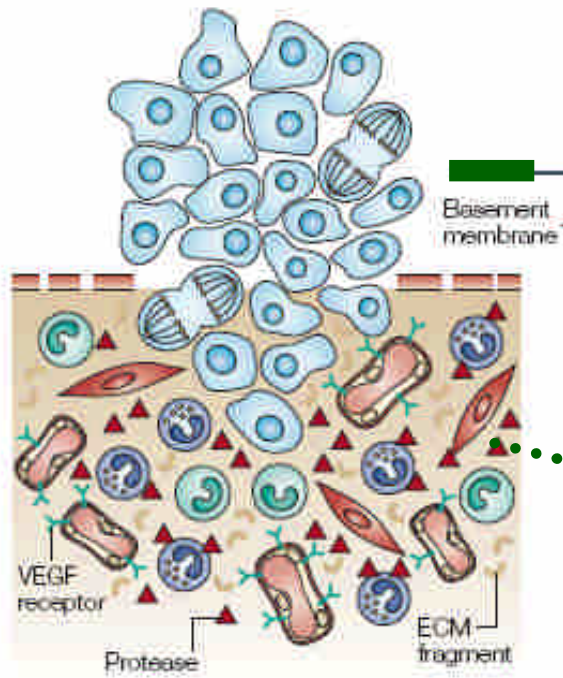
TSP-1 has anti-tumor potentials by inhibiting angiogenesis and stroma reaction during cervical carcinogenesis

Perspectives



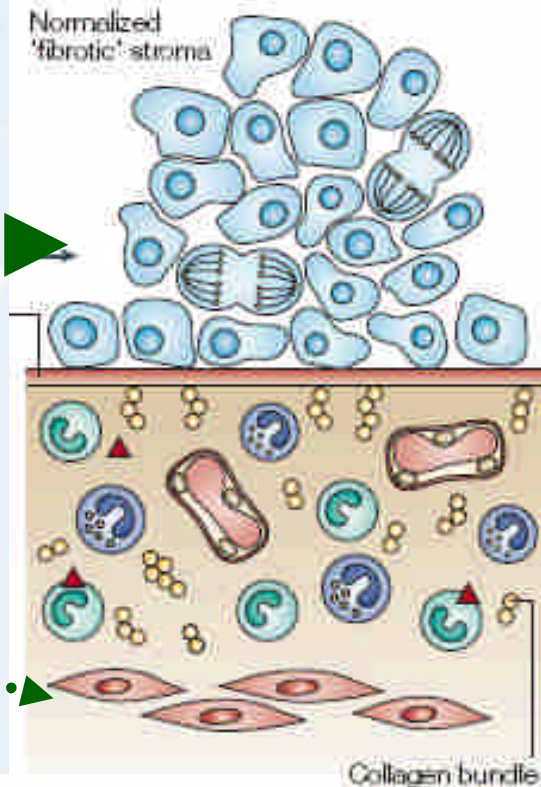
Perspective: Reversion of tumor phenotype by stromal normalization.

a Carcinoma
Reactive tumour stroma:

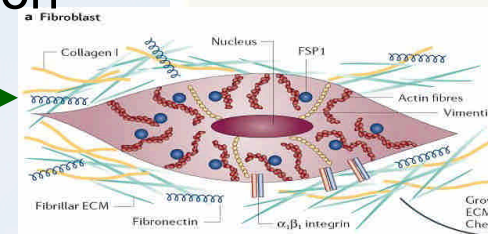
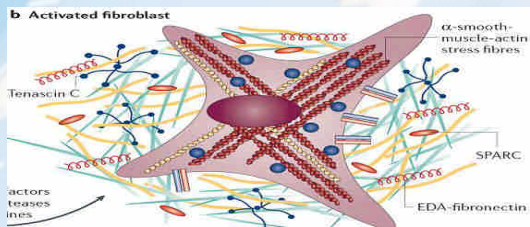


?

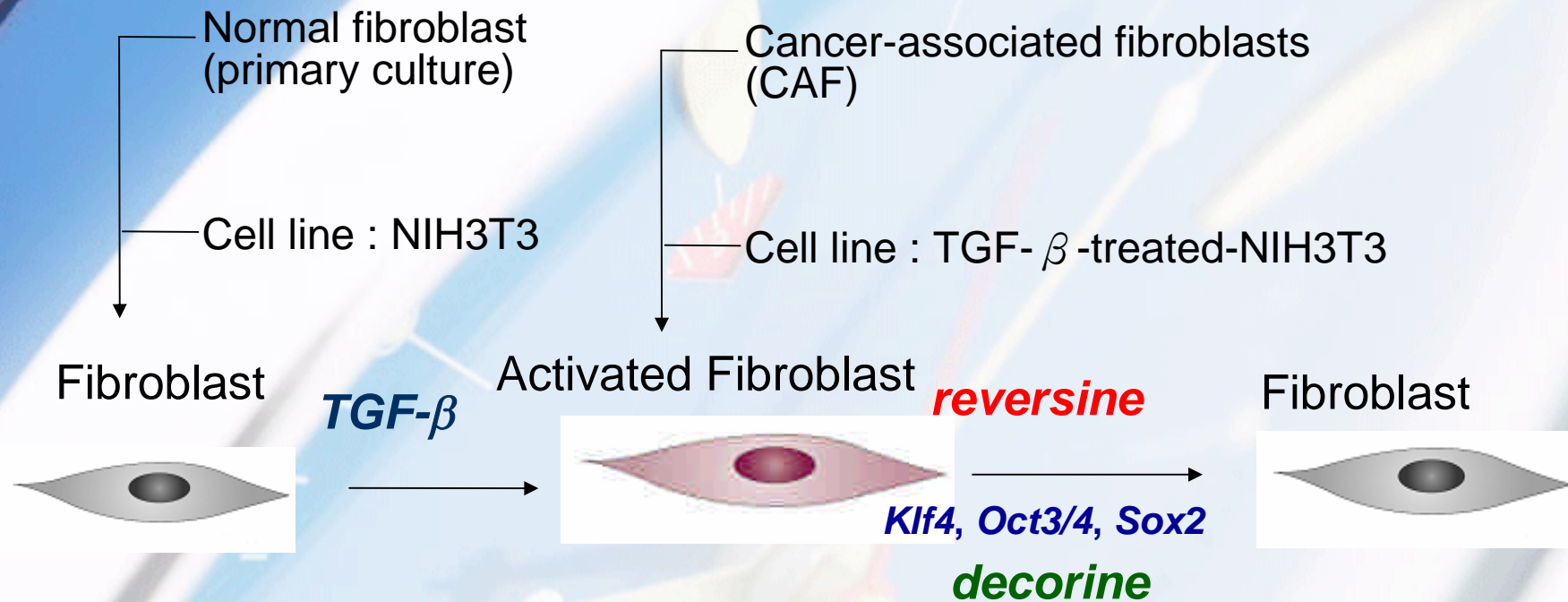
b Pre-malignant dysplasia



de-differentiation

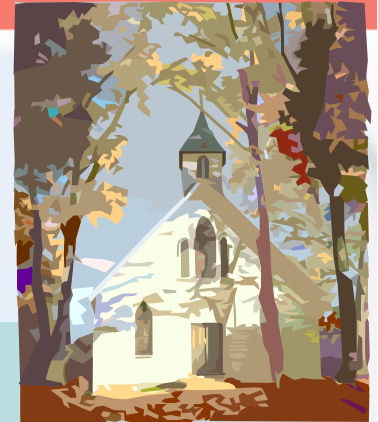


Aim: To de-differentiate myofibroblast into normal fibroblast?



decorine: TGF- β inhibitor \ominus

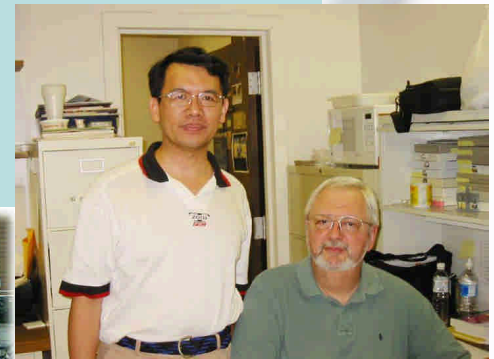
Perspectives & Future directions



1. To revert myofibroblast into normal fibroblasts by de-differentiation?
 - Trans-differentiation (Normal fibroblast → myofibroblast)
 - De-differentiation (myofibroblast → Normal fibroblast)
2. To elucidate the interaction between TSP-1 and TGF- β →
 - Type 1 repeat (TSR)
3. To elucidate the signal pathways about
 - myofibroblasts migration invasion (N-Cadherin)
 - TSP-1: PI-3K (Watnick et al. 2003 Cancer Cell; Elzie CA 2004 IJBCB) →
 - Fibroblast transdifferentiation
 - TGF- β : SMAD (Derynck R 2001 Nature Genet; Rahimi RA 2007 J Cell Biochem) →
4. The roles of ECM remodeling in pelvic organ prolapse (POP) ? →

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Thank You



2000



2007

I have fought the good fight,
I have finished the race, I have kept the faith
Now there is in store for me the crown of
righteousness

The Holy Bible



(2004.05 -2005.05 國科會千里馬計劃)

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