[4/18/2008(Fri) 15:00~15:35/1st FL)]

Potential drug targets in the GPCR-$G\alpha_{12}/G\alpha_{13}$ signaling pathways

Sang Geon Kim

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GPCRs are large families of cell surface receptors that transmit signals through conformational changes upon ligand activation and an interaction with the heterotrimeric G-proteins. GPCRs regulate the cell-signaling pathways and participate in the regulation of physiological processes through the G-proteins defined by their α subunits. A family of 20 G protein-coupled receptors (GPCRs) that provide a large class of tractable drug targets for new anti-inflammatory drugs and, in certain instances, for the treatment of the inflammatory indications such as atherosclerosis, rhinitis, asthma, pulmonary disease and arthritis. In view of the important findings showing that $G\alpha_{12}/G\alpha_{13}$ regulate the various cellular processes such as actin-stress fiber formation, neurite retraction, platelet aggregation, gene induction, and apoptosis, we became interested in whether, after coupling to the activated GPCRs, the G-proteins and their downstream molecules might be involved in the pathologic processes of chronic inflammatory diseases (e.g., liver fibrosis). In this symposium, the possible link of the G-proteins with the pathophysiology will be discussed with the aim of finding potential new drug targets.

Potential Drug Targets in the GPCR-$G\alpha_{12}/G\alpha_{13}$ Signaling Pathways

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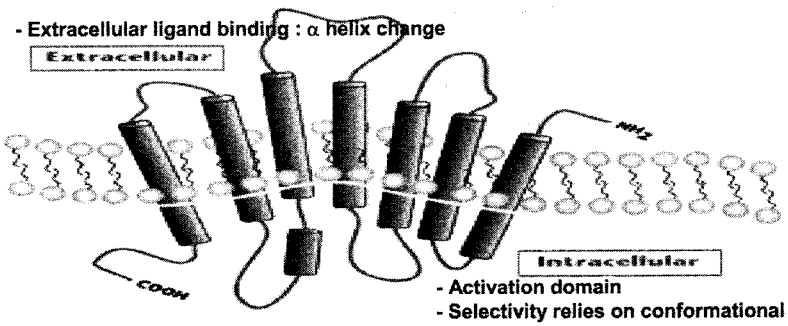
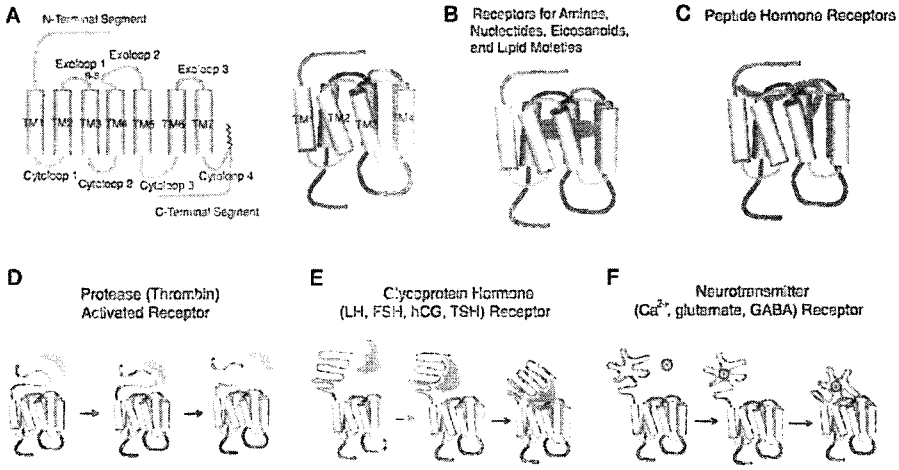
G-protein coupled receptors (GPCRs)



Ligands	Functions
Phospholipids	Growth
Growth factor	Secretion
Neurotransmitter	Neurotransmission
Odorant	Smell
Tastes	Taste
Hormone	Metabolism
Chemoattractant	Immune and inflammation

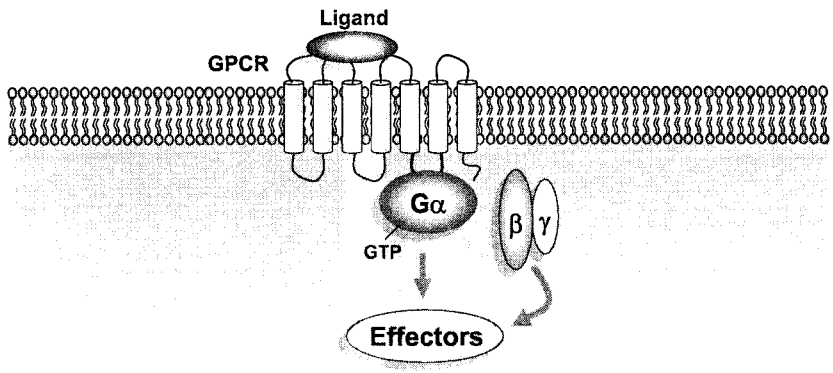
(Stephen et al. *Neurosignal*, 2003)

General structure of GPCRs and receptor-ligand interactions



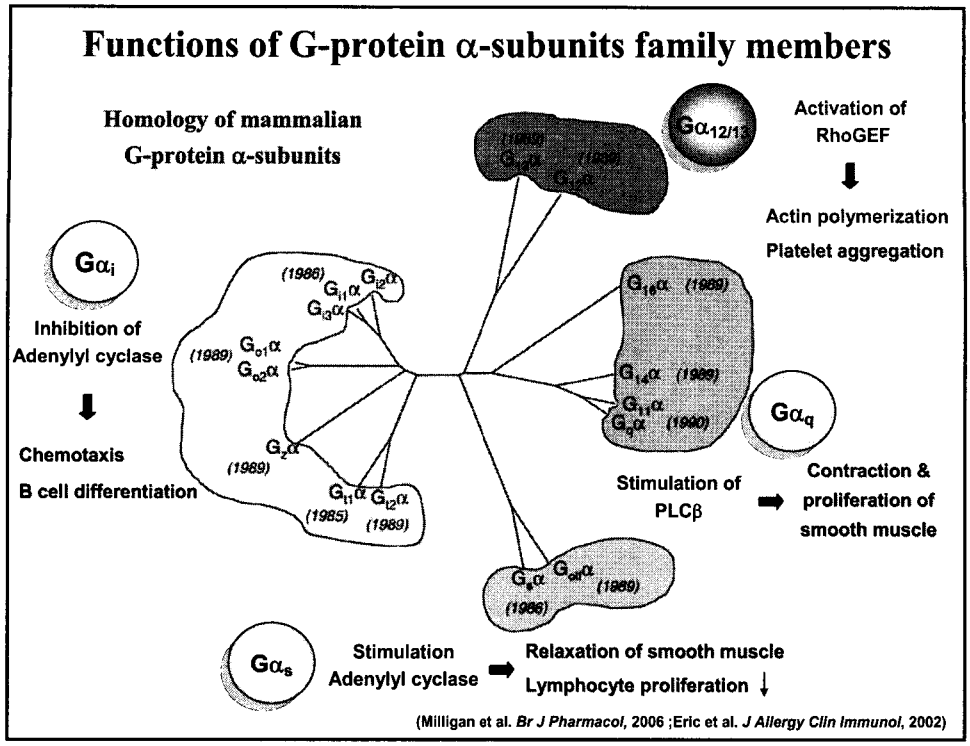
(Ji et al. *J Biol Chem*, 1998)

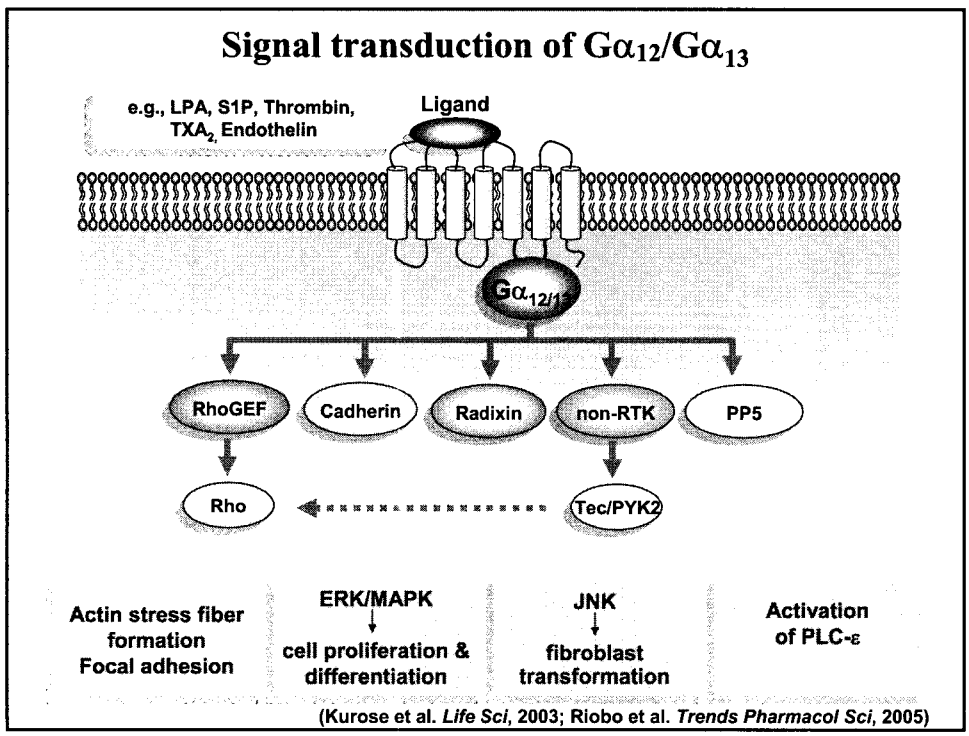
GPCR-G proteins: Signal transduction



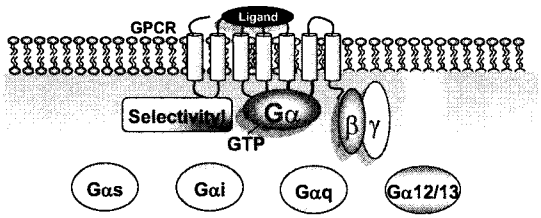
Adenylate cyclases	PLA, PLC	Ion channels	Small GTPases

(Bhattacharya et al. *Biochem Soc Trans*, 2002)


Functions of G-protein α-subunits family members
Homology of mammalian G-protein α-subunits
Gαi
Inhibition of Adenylyl cyclase
Chemotaxis
B cell differentiation
Gαs
Stimulation Adenylyl cyclase
Relaxation of smooth muscle
Lymphocyte proliferation
Gαq
Stimulation of PLCβ
Contraction & proliferation of smooth muscle
Gα12/13
Activation of RhoGEF
Actin polymerization
Platelet aggregation
(Milligan et al. Br J Pharmacol, 2006 ;Eric et al. J Allergy Clin Immunol, 2002)


Signal transduction of Gα12/Gα13
e.g., LPA, S1P, Thrombin, TXA2, Endothelin
Ligand
Gα12/13
RhoGEF
Cadherin
Radixin
non-RTK
PP5
Rho
Tec/PYK2
Actin stress fiber formation Focal adhesion
ERK/MAPK
cell proliferation & differentiation
JNK
fibroblast transformation
Activation of PLC-ε
(Kurose et al. Life Sci, 2003; Riobo et al. Trends Pharmacol Sci, 2005)

GPCR-G protein as drug targets



- ✓ **Their location on the cell surface makes them readily accessible to drugs**
- ✓ **They are also ubiquitous, being involved in regulation of every major mammalian physiological system**
- ✓ **Over 200 major prescription drugs target GPCRs, representing over 30% of the total drugs on the market.**

Top 20 drugs sales (2003) in US$ billions

Drug	Target	Sales
Lipitor	enzyme	5.8
Zocor	enzyme	4.4
Prevacid	enzyme	4.0
Procrit	agonist	3.3
Zyprexa	GPCR	3.2
Epogen	agonist	3.1
Nexium	enzyme	3.1
Zoloft	GPCR	2.9
Celebrex	enzyme	2.6
Neurontin	analgesic	2.4
Advair diskus	GPCR	2.3
Plavix	GPCR	2.2
Norvasc	ion channel	2.2
Effexor XR	SSRI	2.1
Pravachol	enzyme	2.0
Risperdal	GPCR	2.0
Oxycontin	GPCR	1.9
Fosamax	osteoporosis	1.8
Protonix	GPCR	1.8
Vioxx	enzyme	1.8

=> Many of these drugs are amongst the top-selling drugs today including several block-buster drugs

$G\alpha_{12}/G\alpha_{13}$-mediated signaling as potential drug targets

NATURE MEDICINE VOLUME 9 | NUMBER 11 | NOVEMBER 2003

Anti-Platelet drugs

nature medicine

G_{13} is an essential mediator of platelet activation in hemostasis and thrombosis

Alexandra Moers[1], Bernhard Nieswandt[2], Steffen Massberg[3], Nina Wettschureck[1], Sabine Grüner[2], Ildiko Konrad[3], Valerie Schulte[2], Barsom Aktas[2], Marie-Pierre Gratacap[1,5], Melvin I Simon[4], Meinrad Gawaz[3] & Stefan Offermanns[1]

NATURE MEDICINE VOLUME 14 | NUMBER 1 | JANUARY 2008

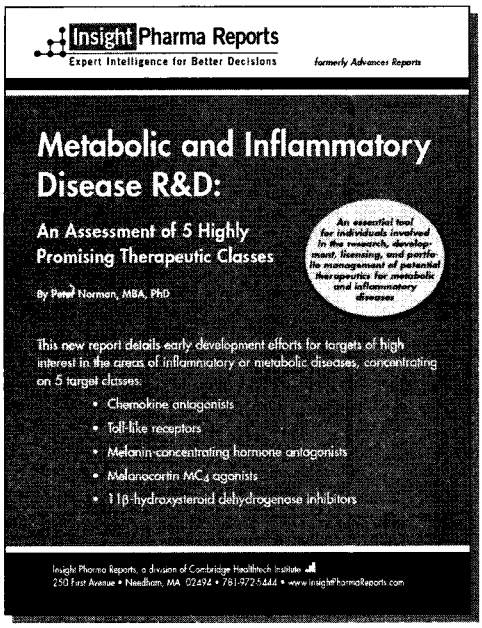
Anti-hypertension drugs

nature medicine

G_{12}-G_{13}–LARG–mediated signaling in vascular smooth muscle is required for salt-induced hypertension

Angela Wirth[1], Zoltán Benyó[1,2,7], Martina Lukasova[1,7], Barbara Leutgeb[1,6], Nina Wettschureck[1], Stefan Gorbey[3], Petra Őrsy[1], Béla Horváth[1], Christiane Maser-Gluth[1], Erich Greiner[4,6], Björn Lemmer[3], Günther Schütz[4], J Silvio Gutkind[5] & Stefan Offermanns[1]

Metabolic and inflammatory disease



Inflammatory Diseases

- Respiratory diseases
- Arthritis

Metabolic Diseases

- Diabetes
- Obesity
- Metabolic Syndrome
- Lipid Disorders

Metabo ... **as highly**

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✓ **Chemok** ... **d receptors**

(GPCRs) 1 ... **new anti-**

inflammat ...

CRs

CCR5

cells

Table 1. CC Family of Chemokines and Chemokine Receptors.*

Receptor	Chemokine Ligands	Cell Types	Disease Connection
CCR1	CCL3 (MIP-1α), CCL5 (RANTES), CCL7 (MCP-3), CCL14 (HCC1)	T cells, monocytes, eosinophils, basophils	Rheumatoid arthritis, multiple sclerosis
CCR2	CCL2 (MCP-1), CCL8 (MCP-2), CCL7 (MCP-3), CCL13 (MCP-4), CCL16 (HCC4)	Monocytes, dendritic cells (immature), memory T cells	Atherosclerosis, rheumatoid arthritis, multiple sclerosis, resistance to intracellular pathogens, type 2 diabetes mellitus
CCR3	CCL11 (eotaxin), CCL13 (eotaxin-2), CCL7 (MCP-3), CCL5 (RANTES), CCL8 (MCP-2), CCL13 (MCP-4)	Eosinophils, basophils, mast cells, Th2, platelets	Allergic asthma and rhinitis
CCR4	CCL17 (TARC), CCL22 (MDC)	T cells (Th2), dendritic cells (mature), basophils, macrophages, platelets	Parasitic infection, graft rejection, T-cell homing to skin
CCR5	CCL3 (MIP-1α), CCL4 (MIP-1β), CCL5 (RANTES), CCL11 (eotaxin), CCL14 (HCC1), CCL16 (HCC4)	T cells, monocytes	HIV-1 coreceptor (T-tropic strains), transplant rejection
CCR6	CCL20 (MIP-3β, LARC)	T cells (T regulatory and memory), B cells, dendritic cells	Mucosal humoral immunity, allergic asthma, intestinal T-cell homing
CCR7	CCL19 (ELC), CCL21 (SLC)	T cells, dendritic cells (mature)	Transport of T cells and dendritic cells to lymph node, antigen presentation, and cellular immunity
CCR8	CCL1 (I309)	T cells (Th2), monocytes, dendritic cells	Dendritic-cell migration to lymph node, type 2 cellular immunity, granuloma formation
CCR9	CCL25 (TECK)	T cells, IgA+ plasma cells	Homing of T cells and IgA+ plasma cells to the intestine, inflammatory bowel disease
CCR10	CCL27 (CTACK), CCL28 (MEC)	T cells	T-cell homing to intestine and skin

Table 2. CXC, CX_3C, and XC Families of Chemokines and Chemokine Receptors.*

Receptor	Chemokine Ligands	Cell Types	Disease Connection
CXCR1	CXCL8 (interleukin-8), CXCL6 (GCP2)	Neutrophils, monocytes	Inflammatory lung disease, COPD
CXCR2	CXCL8, CXCL1 (GROα), CXCL2 (GROβ), CXCL3 (GROγ), CXCL5 (ENA-78), CXCL6	Neutrophils, monocytes, microvascular endothelial cells	Inflammatory lung disease, COPD, angiogenic for tumor growth
CXCR3-A	CXCL9 (MIG), CXCL10 (IP-10), CXCL11 (I-TAC)	Type 1 helper cells, mast cells, mesangial cells	Inflammatory skin disease, multiple sclerosis, transplant rejection
CXCR3-B	CXCL4 (PF4), CXCL9 (MIG), CXCL10 (IP-10), CXCL11 (I-TAC)	Microvascular endothelial cells, neoplastic cells	Angiostatic for tumor growth
CXCR4	CXCL12 (SDF-1)	Widely expressed	HIV-1 coreceptor (T-cell-tropic), tumor metastases, hematopoiesis
CXCR5	CXCL13 (BCA-1)	B cells, follicular helper T cells	Formation of B-cell follicles
CXCR6	CXCL16 (SR-PSOX)	CD8+ T cells, natural killer cells, and memory CD4+ T cells	Inflammatory liver disease, atherosclerosis (CXCL16)
CX_3CR1	CX3CL1 (fractalkine)	Macrophages, endothelial cells, smooth-muscle cells	Atherosclerosis
XCR1	XCL1 (lymphotactin), XCL2	T cells, natural killer cells	Rheumatoid arthritis, IgA nephropathy, tumor response

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Chemokine receptors as drug targets for metabolic and inflammatory disease

INFLAMMATORY DISEASES

Asthma

Chronic obstructive pulmonary disease (COPD)

Rheumatoid arthritis

METABOLIC DISORDERS

Atherosclerosis

INSULIN RESISTANCE AND OBESITY-INDUCED DIABETES

Type 2 diabetes

MULTIPLE SCLEROSIS

Table 3. **Status of Chemokine-Receptor Antagonists in Development.***

Chemokine Receptor	Clinical Indication	Trial Status and Sponsor as of Winter 2005–2006
CCR1	Rheumatoid arthritis	Phase 2, Pfizer Phase 1, Millennium–Aventis
	Multiple sclerosis	Phase 2, Berlex
CCR2	Rheumatoid arthritis	Phase 2b, Millennium Phase 1, AstraZeneca Phase 1, Incyte
	Type 2 diabetes	Phase 1, Incyte
	Multiple sclerosis	Phase 2, Merck Phase 1, Millennium Phase 1, Incyte
CCR3	Allergic rhinitis and asthma	Phase 2, Cambridge Antibody Technology Phase 2, GlaxoSmithKline
CCR5	HIV	Phase 3, Pfizer Phase 2, Schering-Plough
CCR9	Inflammatory bowel disease	Phase 2, ChemoCentryx
CXCR1, CXCR2	Chronic obstructive pulmonary disease	Phase 1, GlaxoSmithKline
CXCR3	Psoriasis	Phase 2, Tularik–Amgen
CXCR4	Stem-cell mobilization	Phase 3, AnorMED

(Charo et al., *N Engl J Med.*, 2006; Palmqvist et al., *Br J Pharmacol.*, 2007; Donnelly et al., *Trends Pharmacol Sci.*, 2006)

Chemokine receptor and $G\alpha_{12}/G\alpha_{13}$-mediated signaling

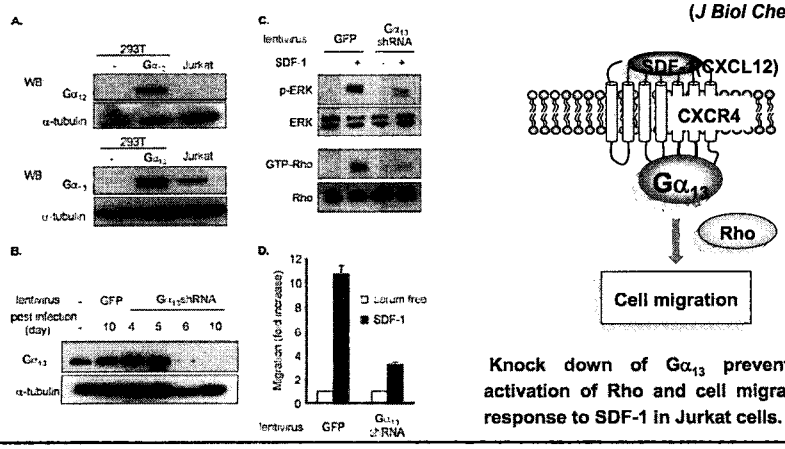
The $G\alpha_{13}$-Rho Signaling Axis Is Required for SDF-1-induced Migration through CXCR4*

Received for publication, September 25, 2006 Published, JBC Papers in Press, October 20, 2006, DOI 10.1074/jbc.M609062200

Wenfu Tan, Daniel Martin, and J. Silvio Gutkind[1]

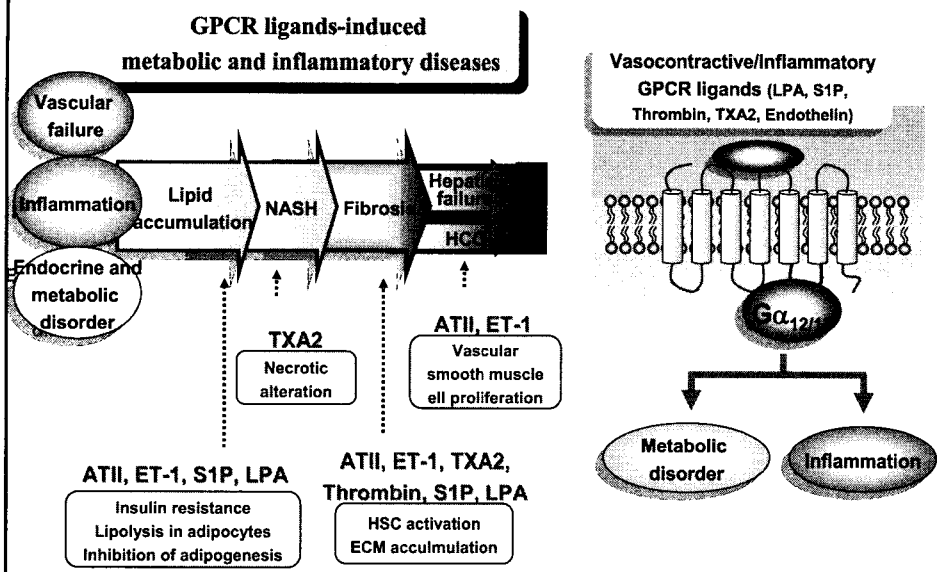
From the Oral and Pharyngeal Cancer Branch, NIDCR, National Institutes of Health, Department of Health and Human Services, Bethesda, Maryland 20892-4330

(*J Biol Chem.*, 2006)

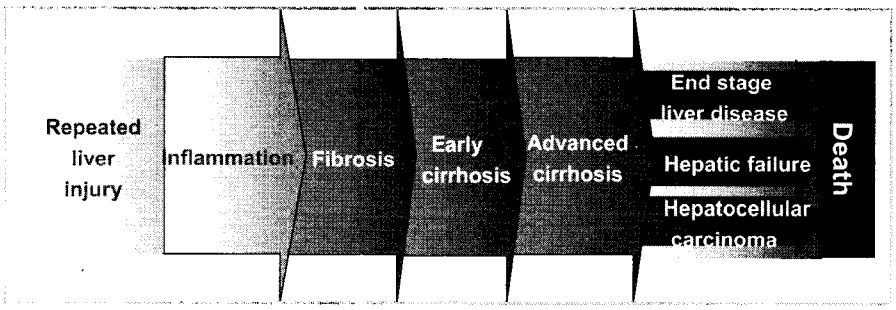


Knock down of $G\alpha_{13}$ prevents the activation of Rho and cell migration in response to SDF-1 in Jurkat cells.

$G\alpha_{12}/G\alpha_{13}$-mediated signaling as potential drug targets for metabolic and inflammatory disease



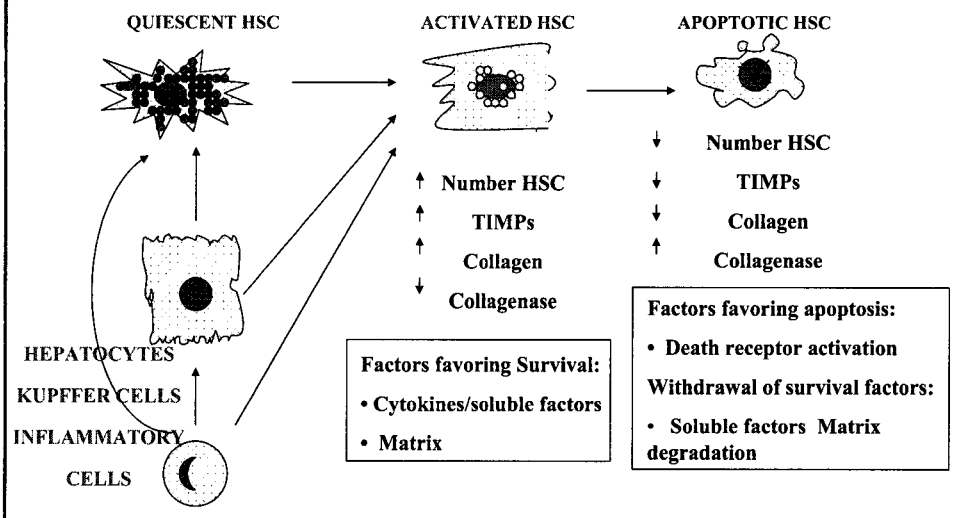
Liver Fibrosis



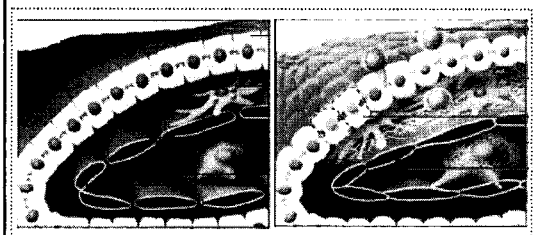
	Death (in thousands, 2002)			
	Cirrhosis	**Liver cancer**	HBV infection	HCV infection
South-east Asia	**204**	**61**	37	14
America	**105**	**37**	6	7
Worldwide	**788**	**618**	104	52
	1,406		156	

FIBROGENESIS

PROGRESSION RESOLUTION



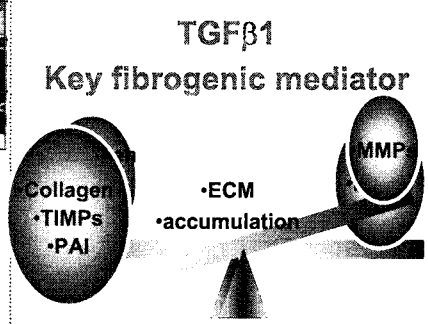
TGFβ1 in liver fibrosis



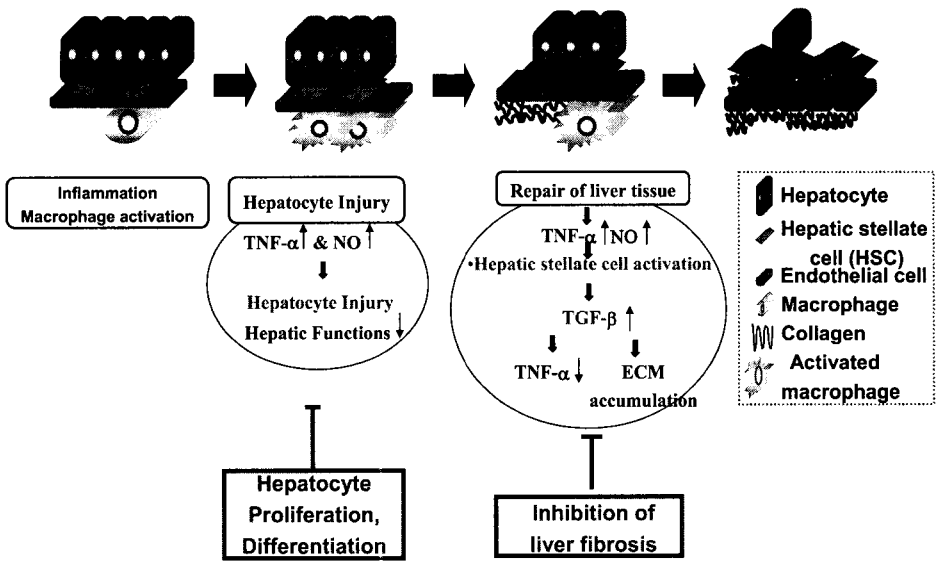
Quiescent HSC
- HGF production

Activated HSC
-Shape change
- Accumulated in perisinusoids
- α-SMA expression
- Induction of TGFβ1
- Collagen synthesis

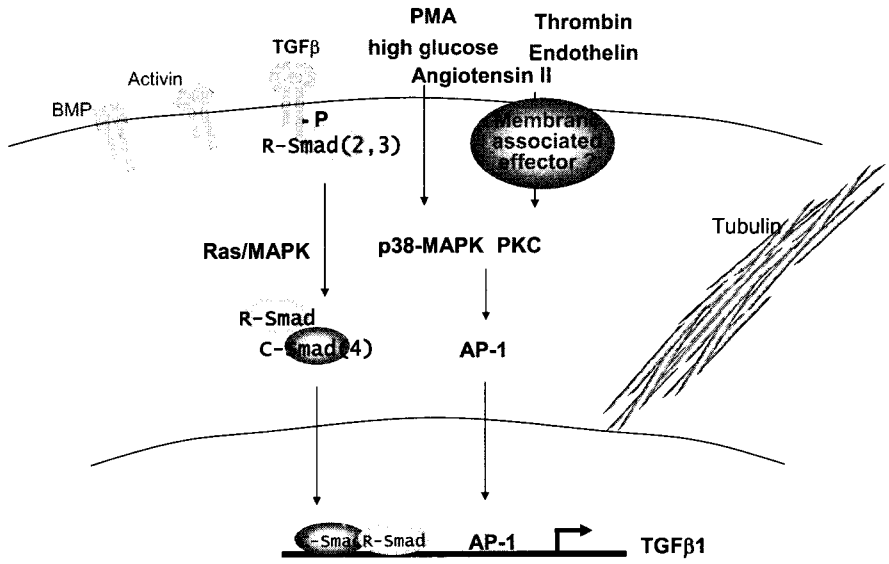
•*Science & Medicine* (S28)



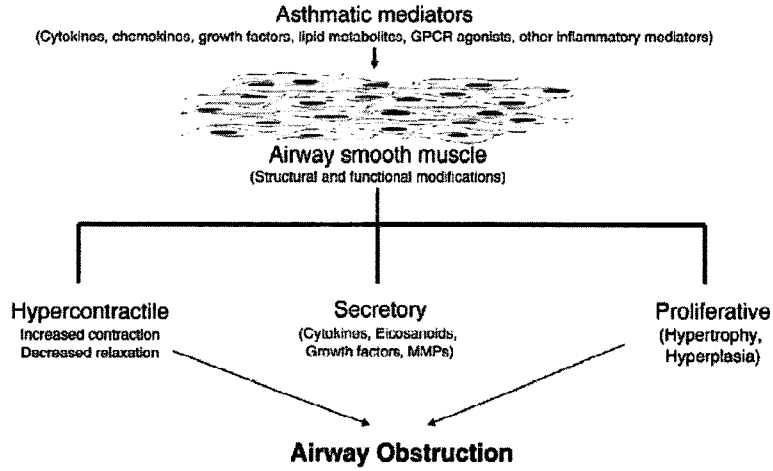
Pathophysiology of liver fibrogenesis



Molecular Mediators for TGFβ1 Regulation



Proposed role of airway smooth muscle in asthma pathogenesis



(Deshpande et al., *Cellular Signaling*, 2006)