新規創薬ターゲットの同定

～創薬におけるChemical Biology～

味の素製薬株式会社

辻　尚志

2013.2.12

本日の内容

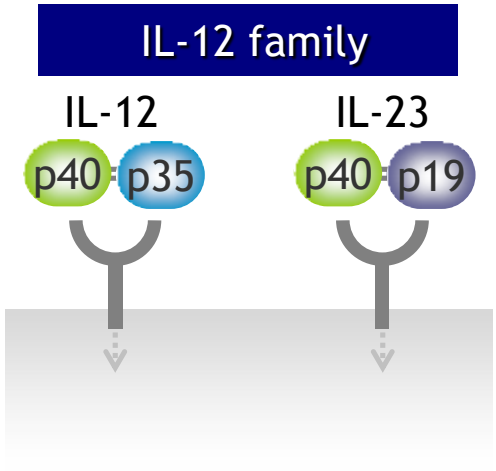
1. はじめに
2. 創薬標的の同定①　PIKfyve
3. 創薬標的の同定②　$G_{\beta\gamma}$
4. 振り返り

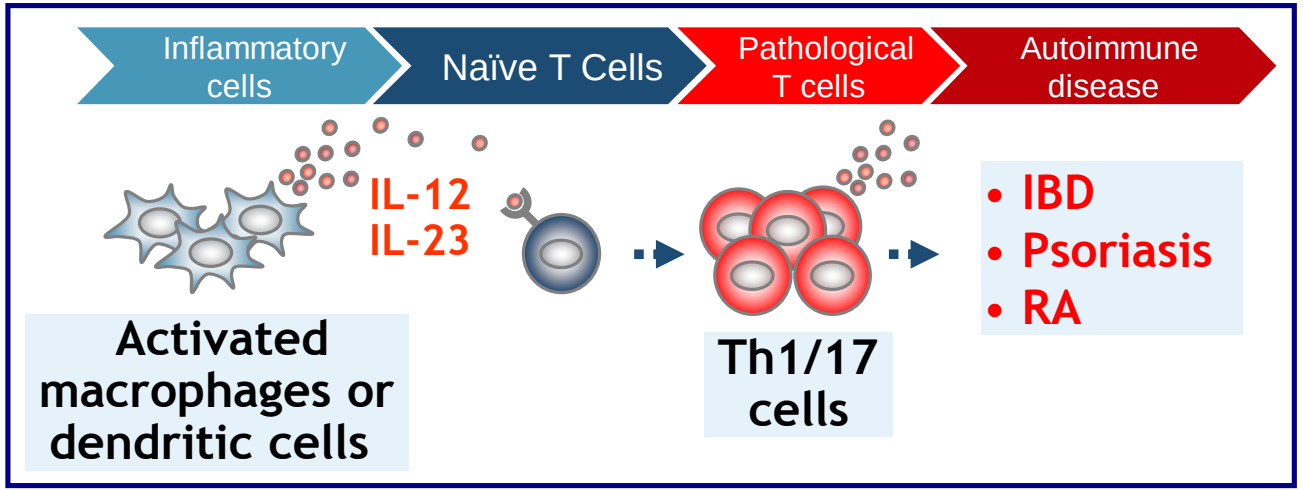
はじめに

- 標的分子が未知の探索研究
  - バリデートされた創薬標的の枯渇？
  - Cell free assay/HTSの限界
  - フェノタイプスクリーニングへ
- 標的分子が捕まるか？
  - 産総研との共同研究
  - 社内での取り組み
- 標的同定後の展開
  - 華々しい展開はあったのか？

① PIKfyve

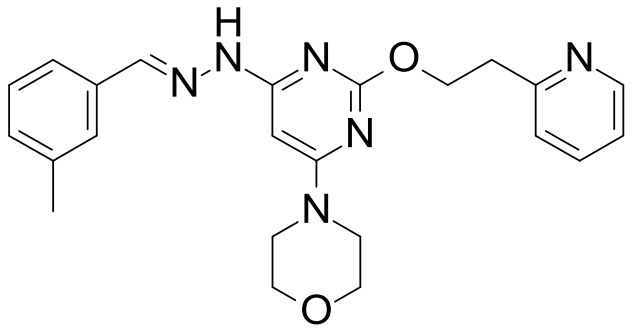
IL-12/23 is validated target for autoimmune diseases





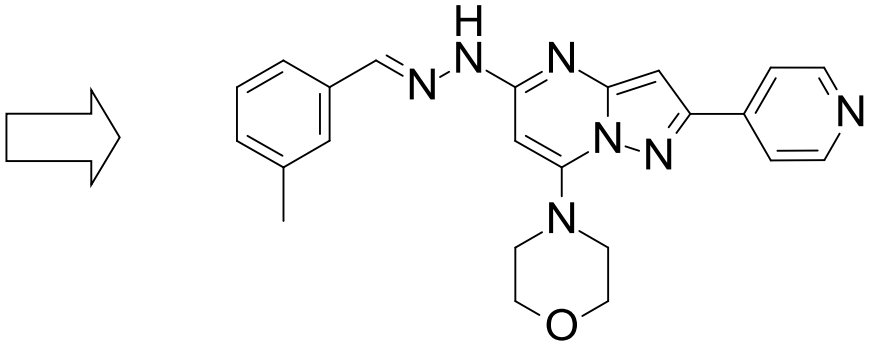
- **Interleukin-12 and 23 (IL-12/23):**
  - **hetero-dimeric pro-inflammatory cytokines sharing p40 subunit**
  - **differentiation and proliferation of Th1/17**
  - **involvement in diverse of autoimmune/inflammatory diseases**
- **Ustekinumab:**
  - **human monoclonal antibody for IL-12p40**
  - **approved for moderate to severe plaque psoriasis in Japan, US, EU and Canada**
  - **promising results in phase 2 clinical trials for Crohn's disease**

APY0201 was discovered by phenotypic cell-based assay



STA5326

- **PII clinical trial was ongoing**
- **mouse TG-PEC cells**
  IL-12p70: IC_{50} = 10 nM



APY0201

mouse TG-PEC cells

IL-12p70: IC_{50} = 8.4 nM
IL-12p40: IC_{50} = 16 nM
TNF-α: IC_{50} > 10000 nM

human PBMC

IL-12p70: IC_{50} = 8.4 nM
IL-12p40: IC_{50} = 99 nM
TNF-α: IC_{50} > 10000 nM

Mouse PK

IV (3 mg/kg)

CL_{tot} = 1.0 L/hr/kg

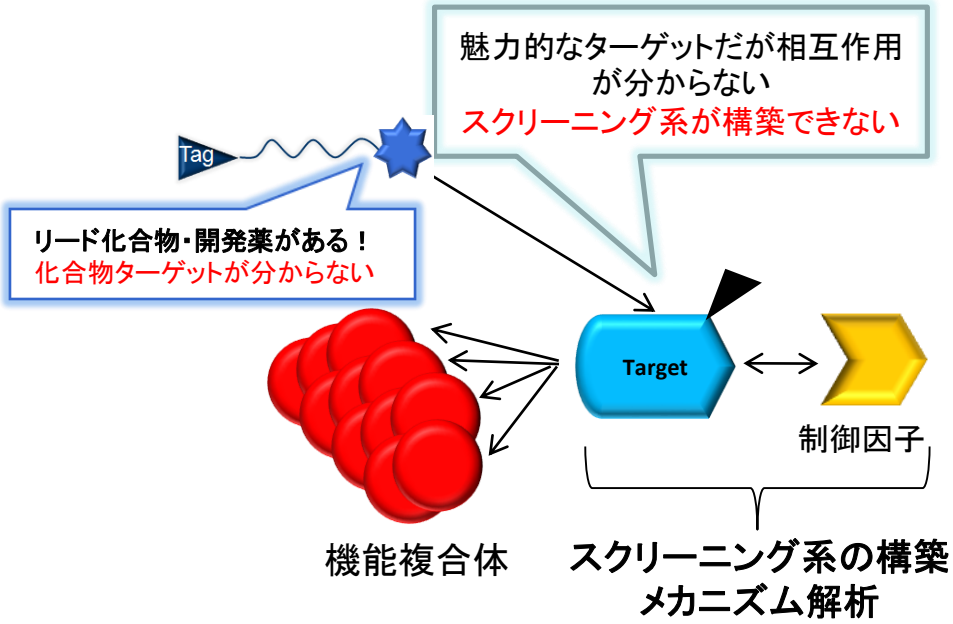
PO (30 mg/kg)

AUC = 12.3 μg·hr/mL
BA = 52 %

Hayakawa N., Andou, A., Yamamoto T *et al. manuscript submitted*

ケモバイオPJ（JBiC）における夏目チームのコアテクノロジーとアクティビティ

製薬企業との課題解決型連携

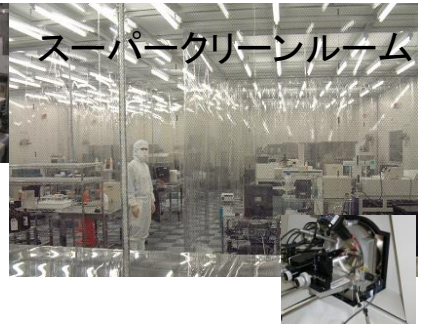


astellas Leading Light for Life
武田薬品工業株式会社
KYOWA KIRIN 協和発酵キリン株式会社
AJINOMOTO
Daiichi-Sankyo 第一三共株式会社
田辺三菱製薬
TAIHO 大鵬薬品
Kowa 興和創薬株式会社
日本化薬
旭化成ファーマ株式会社

基盤技術開発

超高感度質量分析


ロボットによる自動化

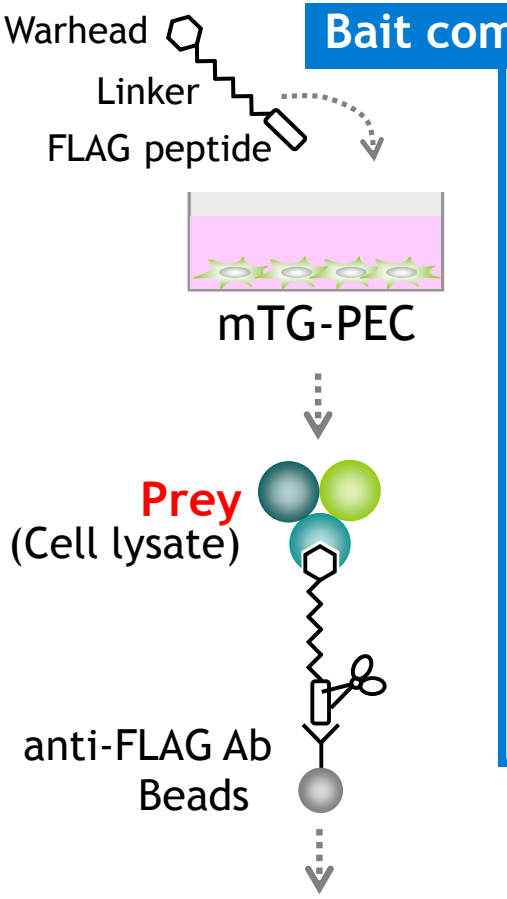


精密電鋳流路によるナノ・フロー

高感度・低ノイズ・高い再現性

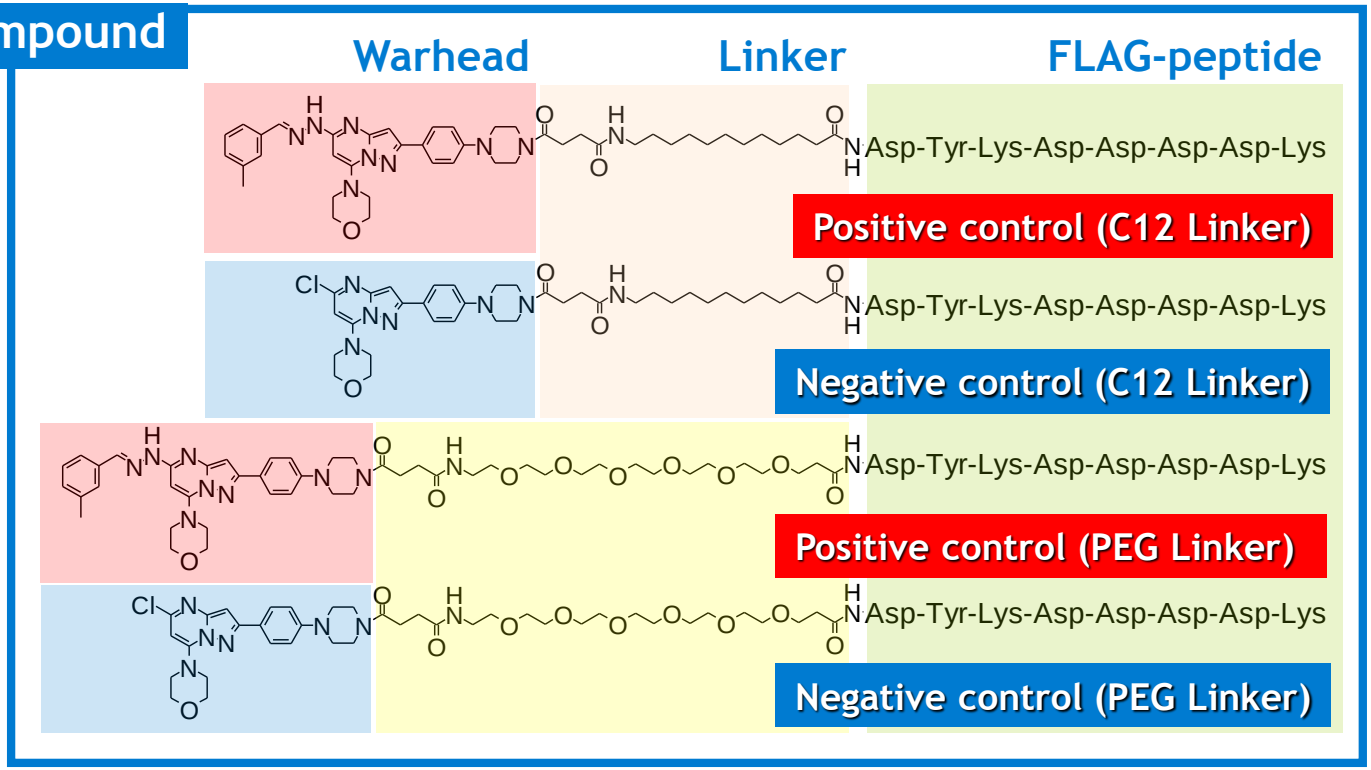
NEDO生物システム制御基盤技術開発
2010年度で終了

Chemical proteomics approach for target identification



Binding protein Identification (direct-nanoflow LC-MS/MS)

Bait compound



Bait compounds were designed and synthesized for the adopted chemical proteomics approach

Vac14 was observed by chemical proteomics approach

#	Gene symbol	Protein name	Bait compounds				Score
			C12-linker		PEG-linker		
			Posi	Nega	Posi	Nega	
1	VAC14	Vac14 homolog	○	×	○	×	4
3	ANXA2	annexin A2	○	×	×	×	3
4	AHNAK	AHNAK nucleoprotein; isoform 1	○	×	○	○	3
5	JAK1	Janus kinase 1	○	○	○	×	3
6	ACTA2	alpha 2 actin	○	×	×	×	3
7	ARPC5	actin related protein 2/3 complex subunit 5	○	×	×	×	3
8	FECH	ferrochelatase	○	×	×	×	3
9	H3F3A	histone family	○	×	×	×	3
10	MTHFD1L	methylenetetrahydrofolate dehydrogenase	○	×	×	×	3
11	MYO1F	myosin IF	○	×	×	×	3
14	CPT1A	carnitine palmitoyltransferase 1A liver	×	×	○	×	3
15	PHB	prohibitin	×	×	○	×	3
16	SGPL1	Sphingosine-1-phosphate lyase 1	×	×	○	×	3
17	VDAC1	voltage-dependent anion channel 1	×	×	○	×	3
18	VDAC1/3/4P	voltage-dependent anion channel 1 or 3 or 4	×	×	○	×	3
19	VDAC2	voltage-dependent anion channel 2	×	×	○	×	3
20	VDAC3	voltage-dependent anion channel 3	×	×	○	×	3

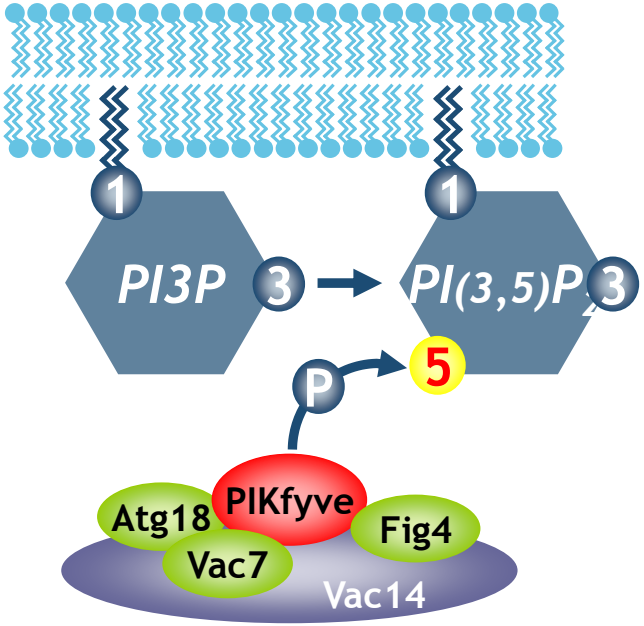
Only VAC14 homolog was found with the two positive bait compounds, but not with two negative ones

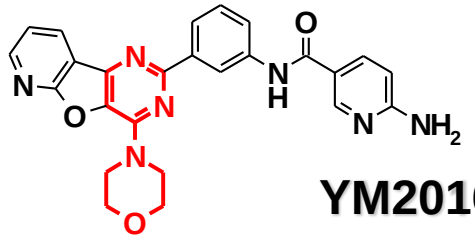
Basic information of Vac14 and PIKfyve kinase

- **Vac14 is a scaffold and activator of PIKfyve kinase**

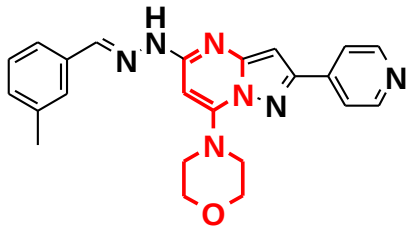
- **PIKfyve kinase:**
  - **lipid kinase acting on PI3P to generate PI(3,5)P_2**
  - **single isoform, ubiquitously-expressed**
  - **immunological function of Vac14 and PIKfyve is totally unknown**

- **YM201636 is an available PIKfyve kinase inhibitor and shares morpholino-pyrimidine moiety with APY0201**





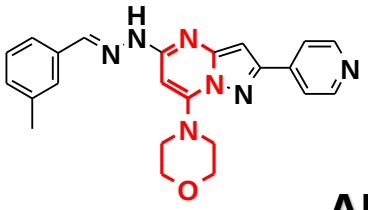
YM201636



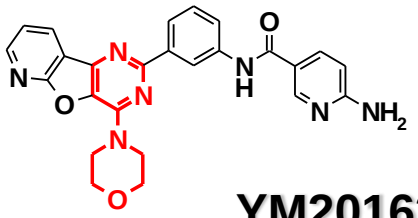
APY0201

APY0201 is a potent and selective PIKfyve kinase inhibitor

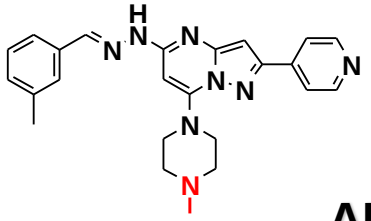
compound	IL-12p70 production in mTG-PEC IC_{50} (nM)	PIKfyve Kinase assay IC_{50} (nM)
APY0201	8.4 nM	5.2 nM
YM201636	2200 nM	148 nM
APY0205	6300 nM	> 1000 nM



APY0201



YM201636

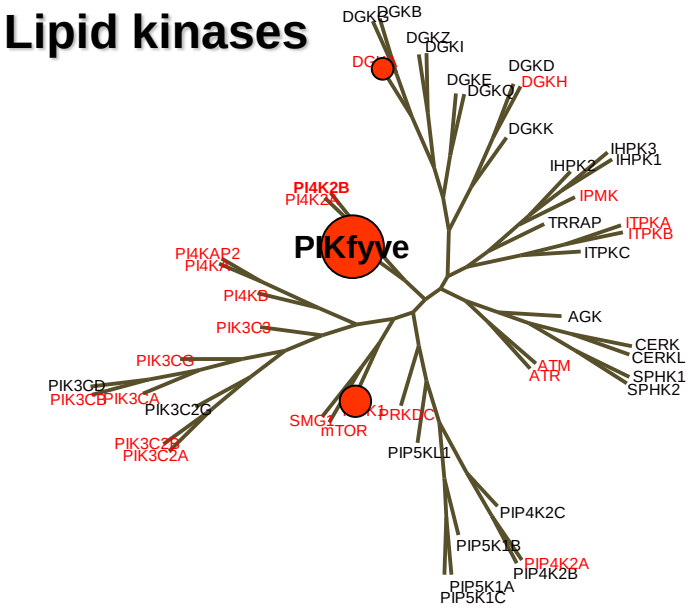


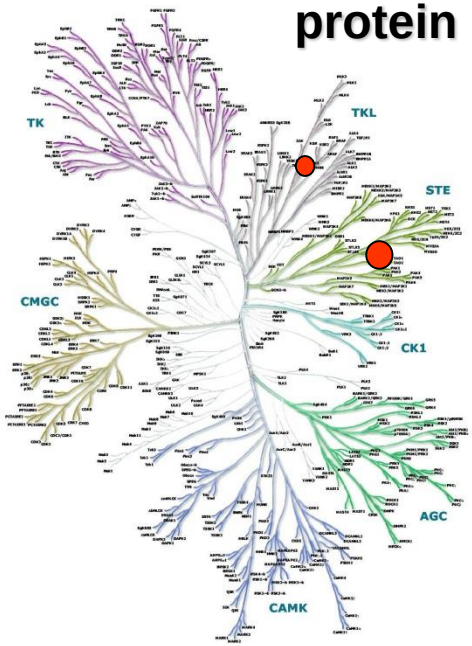
APY0205

IL-12 inhibition -PIKfyve inhibition is closely connected

APY0201 is a potent and selective PIKfyve kinase inhibitor

- **Kinase panel for 24 lipid kinases and for 83 protein kinases:**
  - **Only two kinases inhibited > 50% @300nM: ITPK1 (57%), LOK (56%)**
  - **With an ATP-acylphosphate probe, ATP-competitive inhibition of APY0201 at PIKfyve kinase was confirmed**

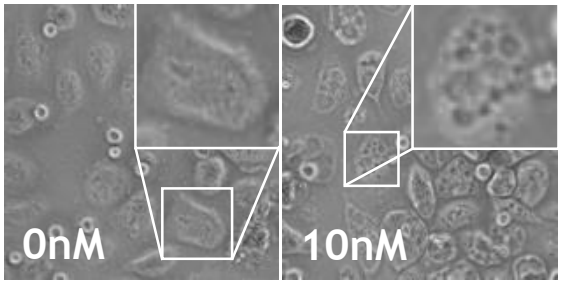




- **Cerep RBA for 137 GPCRs, enzymes, ion channels and transporters:**
  - **No significant inhibition @10μM**

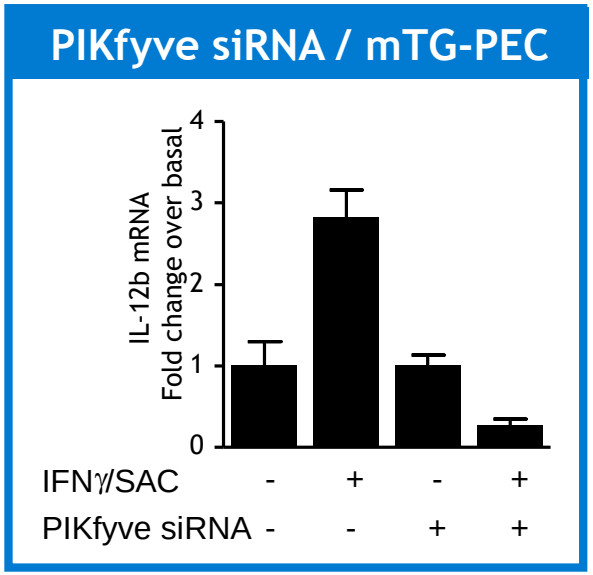
PIKfyve inhibition leads to IL-12 production inhibition

- Cellular vacuolation is induced by PIKfyve inhibition through reduction of $PI(3,5)P_2$.
  - **YM201636 and PIKfyve siRNA induce vacuolation**
  - **APY0201 also showed reversible vacuolation**



- YM201636 inhibits the production of IL-12p70.
  - **IC_{50} = 459 nM (mTG-PEC)**

- PIKfyve siRNA reduced level of IL-12b mRNA, a regulatory gene for IL-12p40.



manuscript ready for submission

① PIKfyve

PIKfyveはDruggable Targetか？

- KOマウスは胎生致死
- Mφをはじめ、全身で空胞化とリンパ球壊死
  - エンドソーム膜の異常？
- ALT上昇
- 体温低下

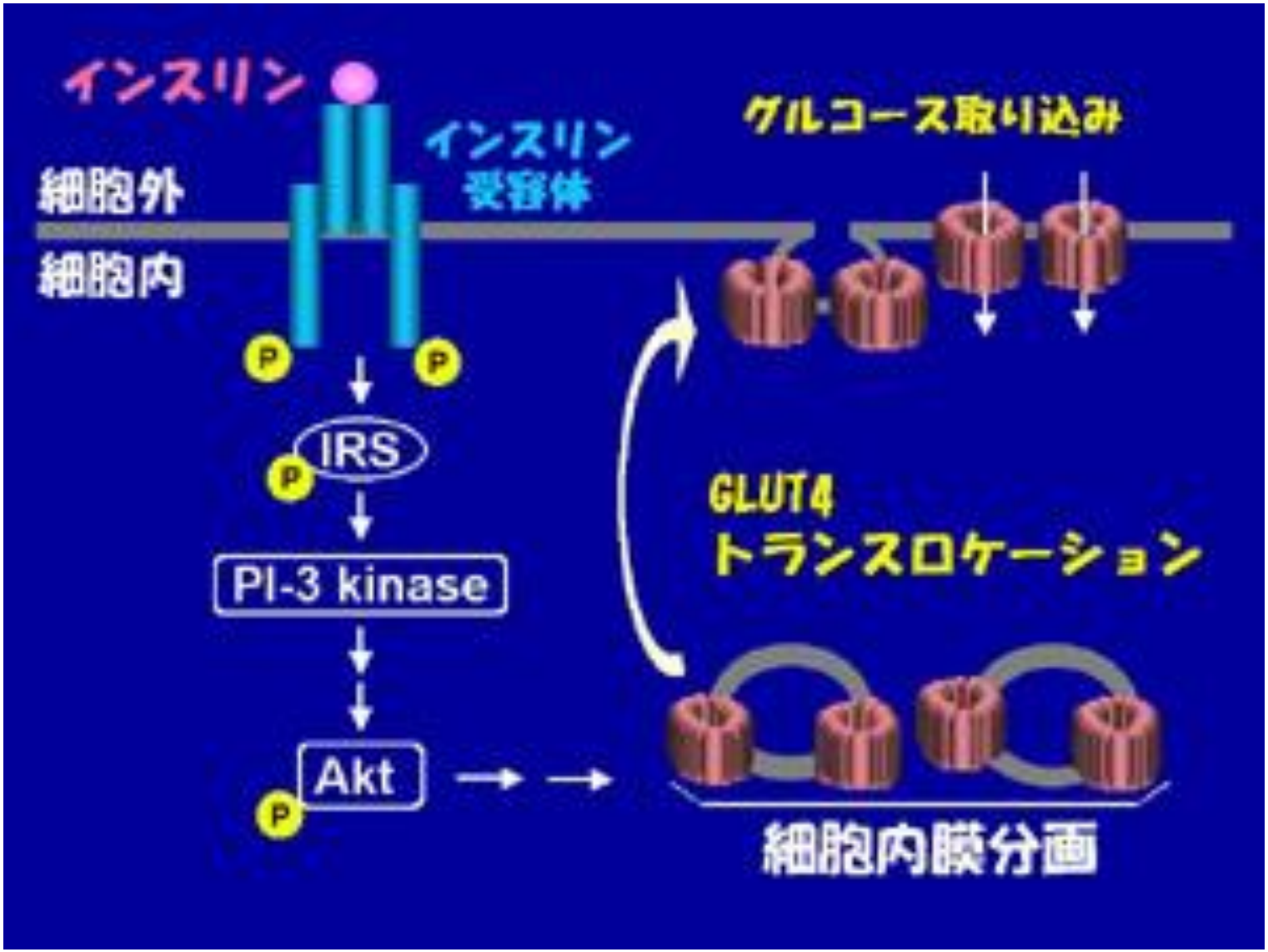
- 臨床では
  - 吐き気、頭痛、めまいなどがDLF

② Gβγ

インスリン様作動薬の探索

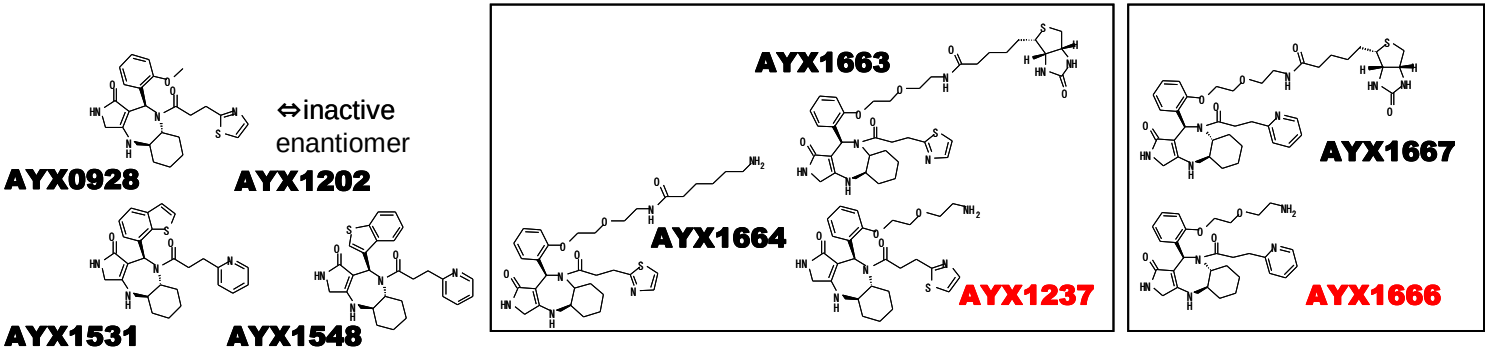
- 細胞レベルでインスリンと同じように糖の取り込みを起こす化合物を得る。

  “経口で服用できるインスリン“
- プライマリーのラット脂肪細胞を用いたスクリーニングを実施。


インスリン
インスリン
受容体
細胞外
細胞内
グルコース取り込み
P
P
IRS
P
PI-3 kinase
Akt
P
GLUT4
トランスロケーション
細胞内膜分画

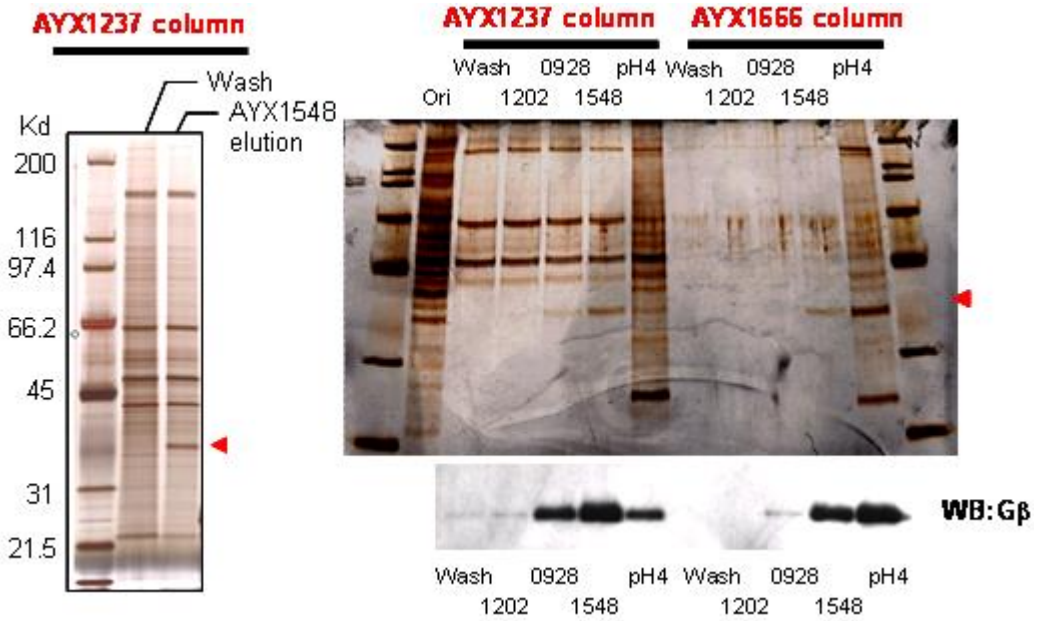
Identification of AJD/AYX binding protein: Gβ

Identification of "binding proteins" using AYX probe molecules.

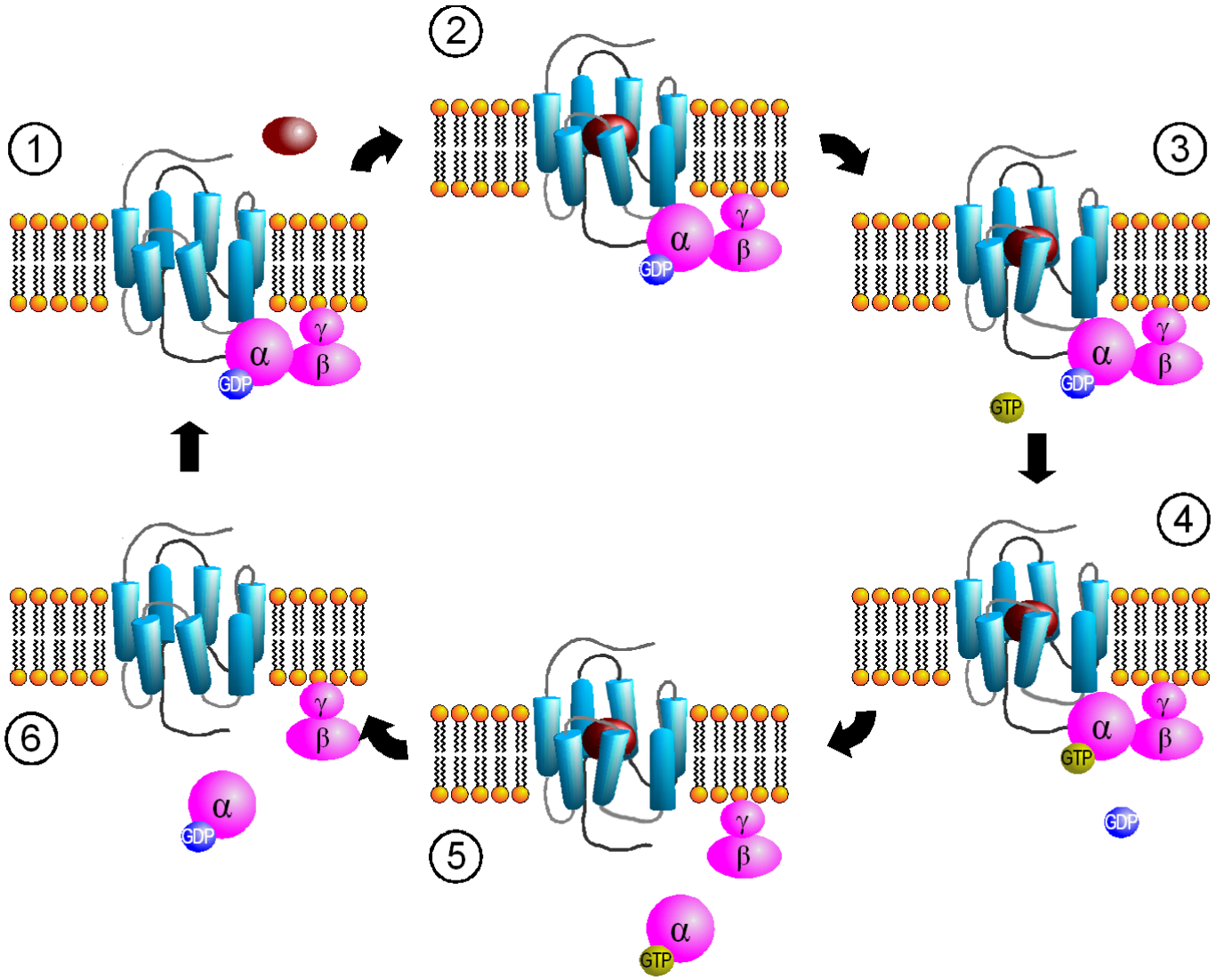


Affinity purification

Affinity choromatography with HLF cells' plasma membrane lysate (0.2% digitonin) yielded 35 kDa protein as specific binding protein to AYX.



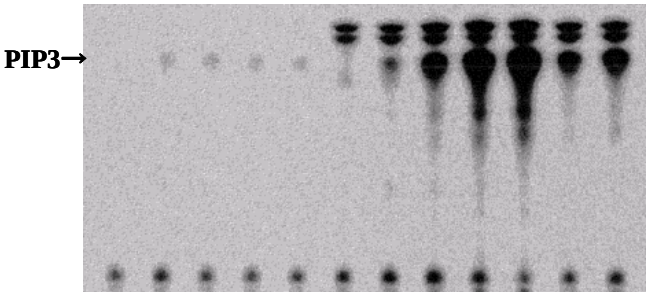
➢**GTP-binding protein β subunit (Gβ) was identified as AJD/AYX binding protein.**


1
2
3
4
5
6
α
β
γ
GDP
GTP

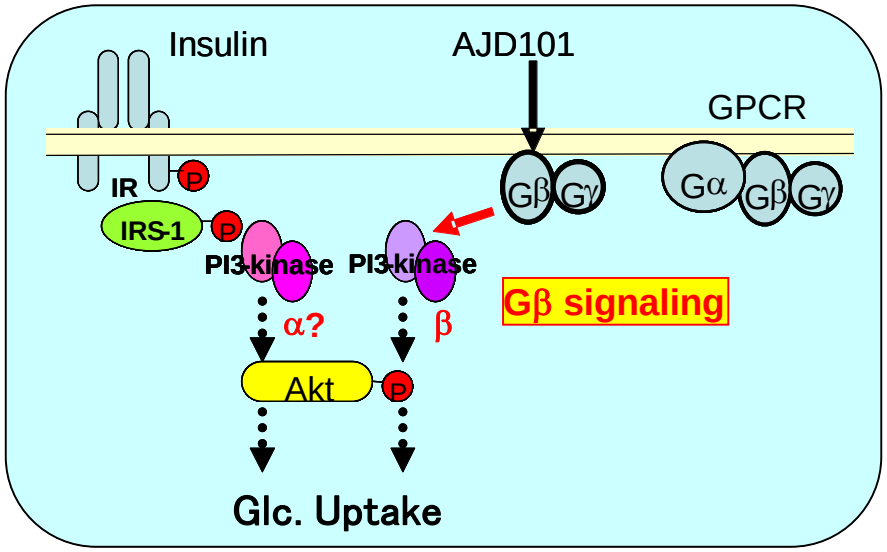
Validation of Gβγ /PI3Kβ

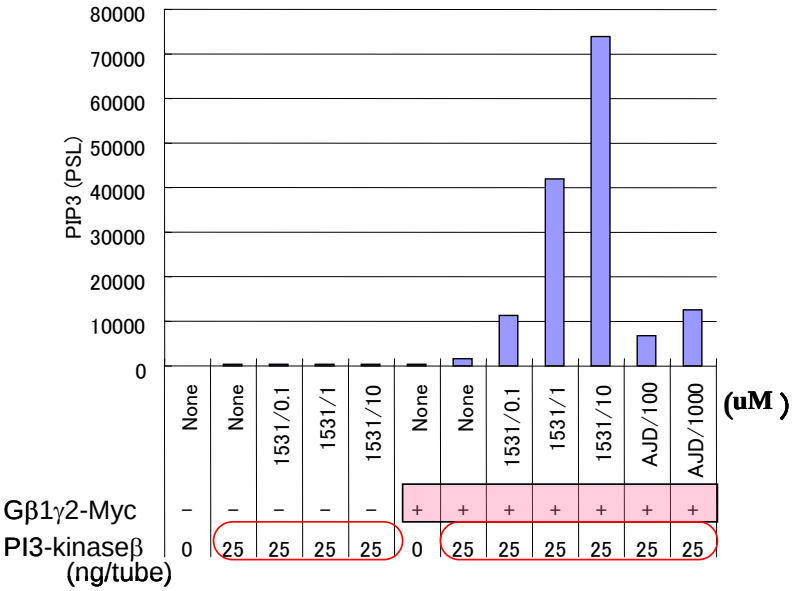
PI3-kinase activation by AJD/AYX. (Cont'd)

Lipid micelle (cont.PtdIns[4,5]P_2)
Gβγ-Myc (Sf21, partial purified)
PI3Kβ (Sf21, Upstate Biotech.)
^{32}P-ATP
+ AJD101 or AYX1531



Schematic Glc. uptake signalings by AJD101/AYX.





➢AJD/AYX enhanced PIP3 production dose-dependently in the presence of PI3-kinase β and Gβγ.

② Gβγ

その後の展開

- 先行品は臨床での効果不十分で中断。
- このコンセプトで強い化合物を得るも、血糖降下作用に結びつかず。
- ターゲットは脂肪細胞か？
- 筋肉組織では別のメカニズム？

振り返り

- 分子標的の同定は何をもたらすか？
  - Go or not go の判断
  - Optimizationの効率化
  - 新規骨格の発見
  - 懲りずにやること

- 分子標的の同定の必要性
  - 開発段階で標的同定は必須
  - 標的同定は開発と平行して進める

1999-2008年にFDAが承認したFirst in Class の低分子新薬50個のうち、28個はﾌｪﾉﾀｲﾌﾟｱｯｾｲから、17個がﾀｰｹﾞｯﾄｱｯｾｲから。

Nature Reviews Drug Discovery,10,507-519（2011）