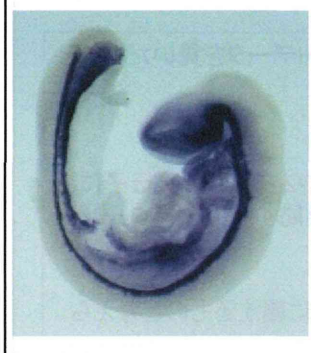
Emage検索によるISHデータ

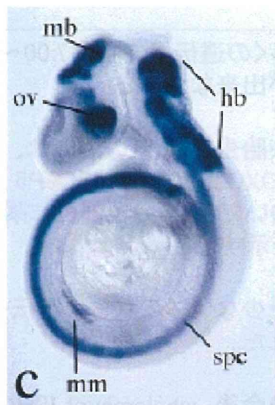
胎生約9.5日胚

Shh sonic hedgehog



Cadm1
cell adhesion molecule 1

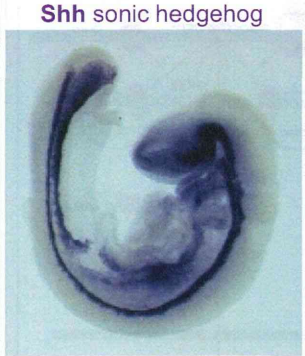
Sfrp2
secreted frizzled-related protein 2



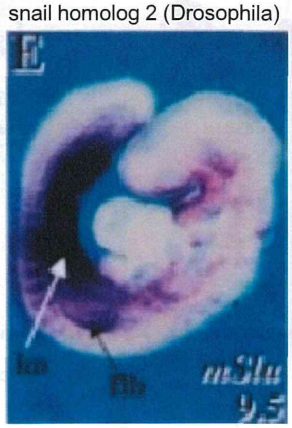
Emage検索によるISHデータ

胎生約9.5日胚

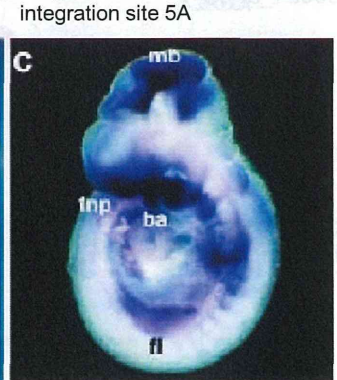
Shh sonic hedgehog

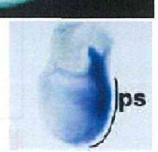


Snai2
snail homolog 2 (Drosophila)



Wnt5a
wingless-related MMTV integration site 5A





発現の空間分布を検討する必要がある

→ Pubmed検索による文献調査あるいは、公開データベースEmage (http://www.emouseatlas.org/emagewebapp/pages/emage_gene_browse.jsf)による検索

多くの遺伝子で胎生7.00～8.50日相当のwholemount ISHデータを見いだすことが出来なかった

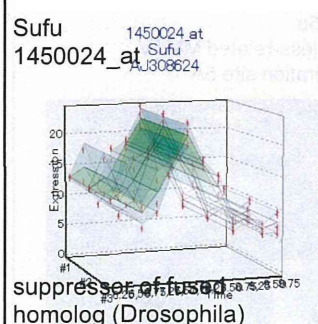
※胎生約9.5日の胚では、
FOXC2、FOXF1、IGFBP5、MEST、MSX1、NCAM1、SFRP2、WNT5A遺伝子について、Shh発現部位に隣接する中胚葉に、空間的に限局する発現パターンを示す

→少なくともこれらの遺伝子はShhシグナルネットワークに属するものと考えられた。

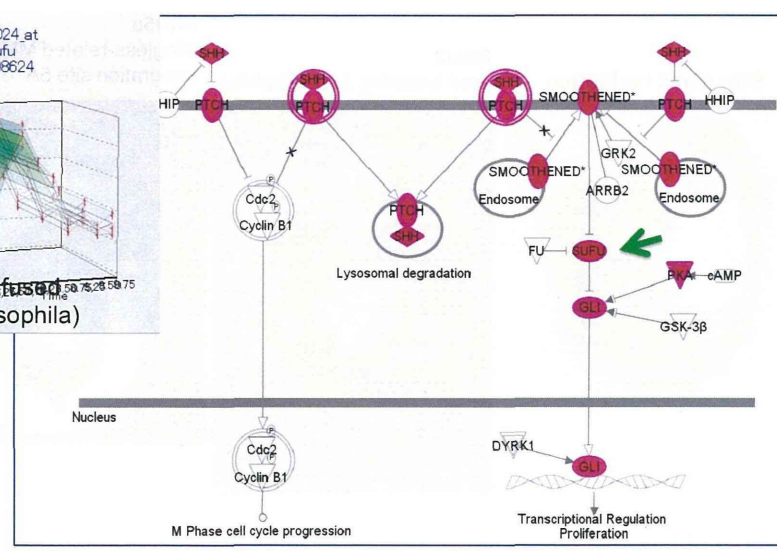
●今後、wholemount ISHの実施による確認作業が必要

抽出遺伝子(648 ps)についての転写制御の解析で37遺伝子が抽出
→37遺伝子以外の遺伝子についてShhシグナルとの関連を文献検索
：Sufu及び Irs1遺伝子

Sufu
1450024_at

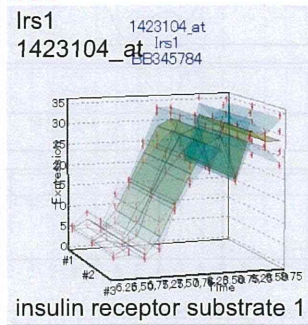


suppressor of fused homolog (Drosophila)



胎生6.25-9.75日で検討したISHデータが見いだせなかった

Irs1



Development 135, 3291-3300 (2008) doi:10.1242/dev.022871

Insulin receptor substrate 1 is an effector of sonic hedgehog mitogenic signaling in cerebellar neural precursors

Susana R. Parathath[1,*], Lori Anne Mainwaring[1,2,*], Africa Fernandez-L[1], Dane Ohlosson Campbell[1] and Anna Marie Kenney[1,2,3,†]

Sonic hedgehog (SHH) and insulin-like growth factor (IGF) signaling are essential for development of many tissues and are implicated in medulloblastoma, the most common solid pediatric malignancy. Cerebellar granule neuron precursors (CGNPs), proposed cells-of-origin for specific classes of medulloblastomas, require SHH and IGF signaling for proliferation and survival during development of the cerebellum. We asked whether SHH regulates IGF pathway components in proliferating CGNPs. We report that SHH-treated CGNPs showed increased levels of insulin receptor substrate 1 (IRS1) protein, which was also present in the germinal layer of the developing mouse cerebellum and in mouse SHH-induced medulloblastomas. Previous roles for IRS1, an oncogenic protein that is essential for IGF-mediated proliferation in other cell types, have not been described in SHH-mediated CGNP proliferation. We found that IRS1 overexpression can maintain CGNP proliferation in the absence of SHH. Furthermore, lentivirus-mediated knock down experiments have shown that IRS1 activity is required for CGNP proliferation in slice explants and dissociated cultures. Contrary to traditional models for SHH signaling that focus on gene transcription, SHH stimulation does not regulate *Irs1* transcription but rather stabilizes IRS1 protein by interfering with mTOR-dependent IRS1 turnover and possibly affects *Irs1* mRNA translation. Thus, we have identified IRS1 as a novel effector of SHH mitogenic signaling that may serve as a future target for medulloblastoma therapies. Our findings also indicate a previously unreported interaction between the SHH and mTOR pathways, and provide an example of a non-classical means for SHH-mediated protein regulation during development.

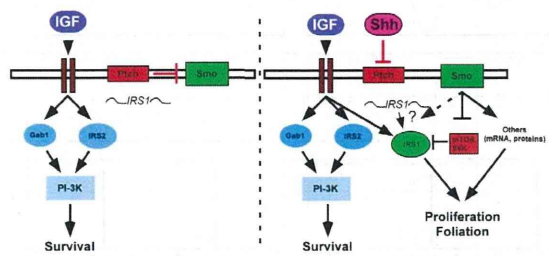
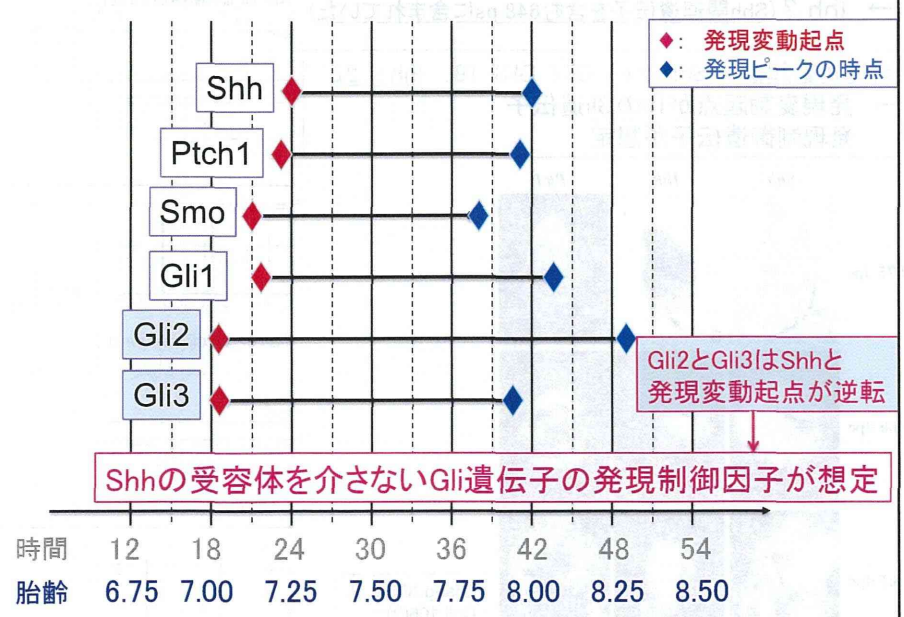
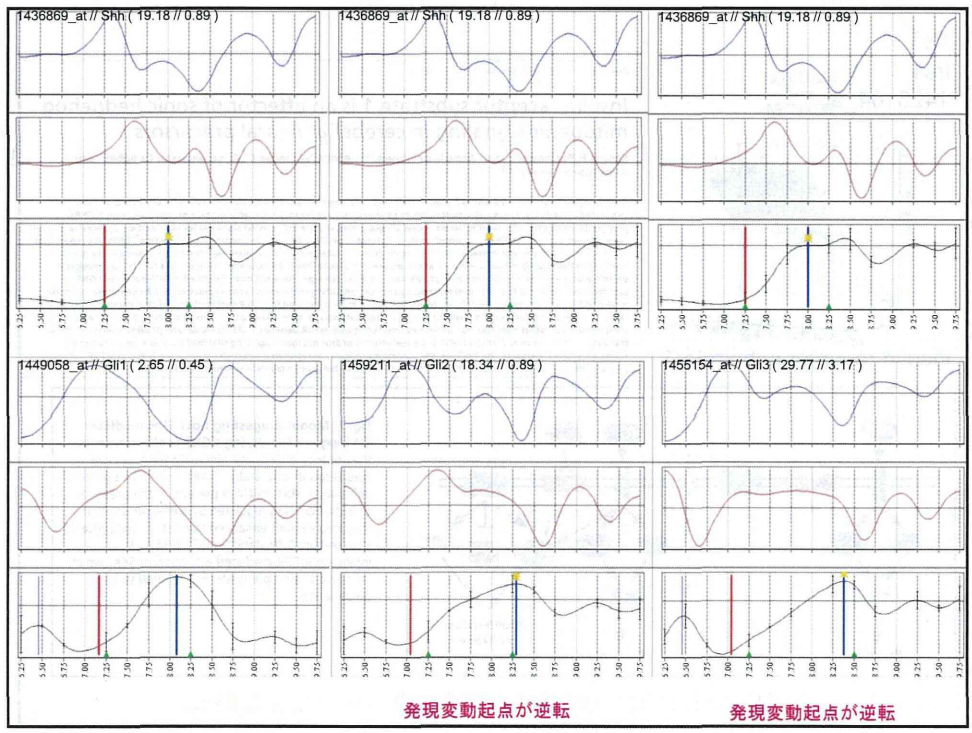


Fig. 7. Model suggesting how SHH mediates IRS1 expression during CGNP proliferation. In the absence of SHH, IGF signaling sends survival cues through IRS2 and/or GAB1 and PI-3K signaling (left panel). IRS1 mRNA is present. In the presence of SHH, IRS1 is upregulated by a mechanism that may involve both enhanced translation and protein stabilization. SHH stabilizes IRS1 protein by inhibiting mTOR-mediated activation of S6K, which is known to phosphorylate IRS1 leading to its degradation.

胎生6.25-9.75日で検討したISHデータが見いだせなかった

Shh関連遺伝子の発現変動起点及び発現ピークの時点



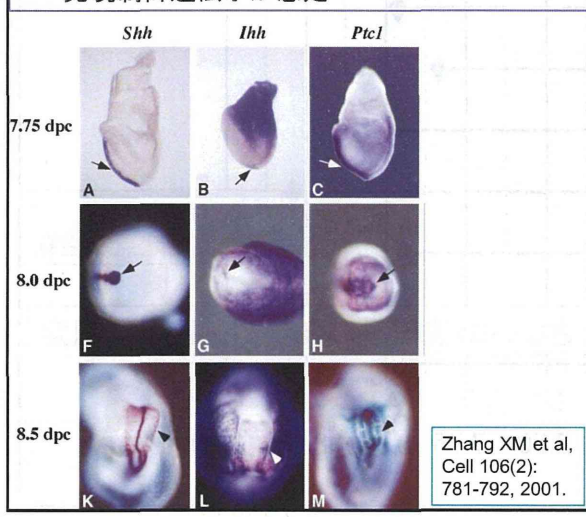


Shhの受容体を介さないGli遺伝子の発現制御因子が想定

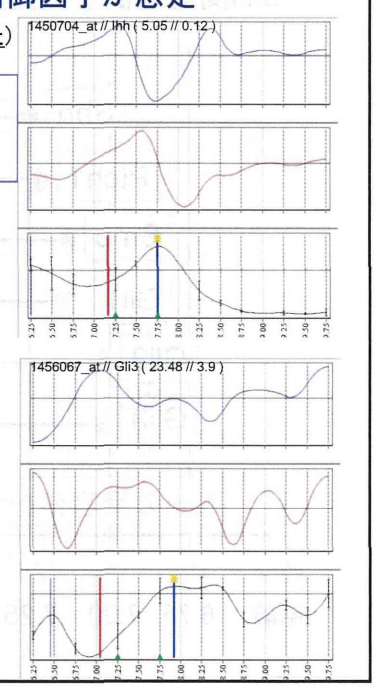
→ Ihh ？(Shh関連遺伝子を含む648 psに含まれていた)

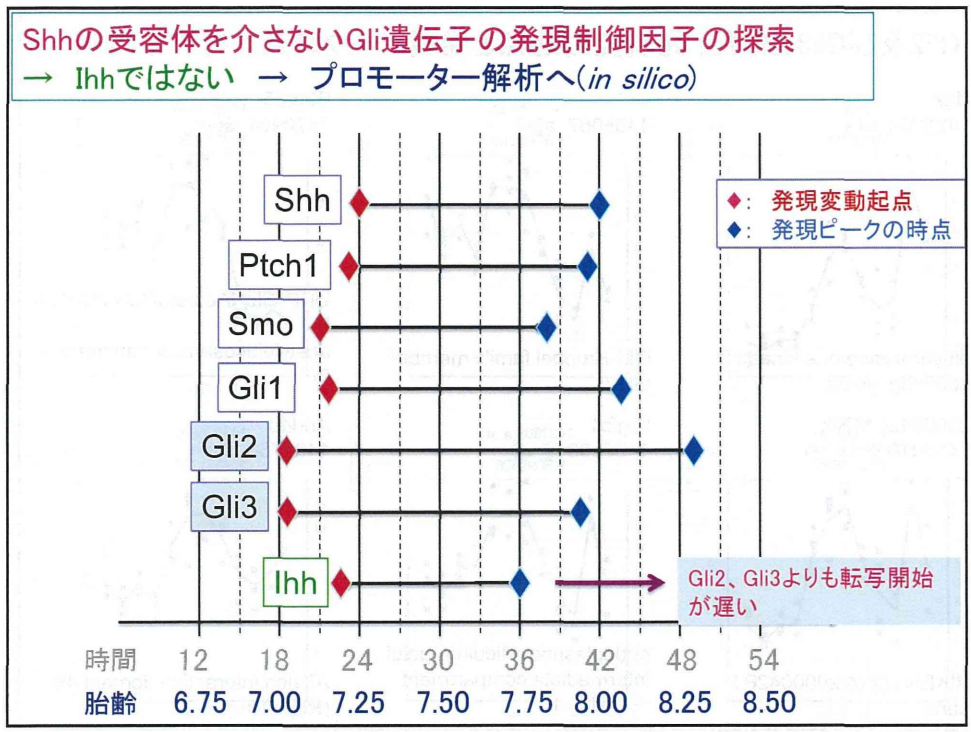
発現変動起点： Shh：24； Gli2, Gli3：19； Ihh： 22

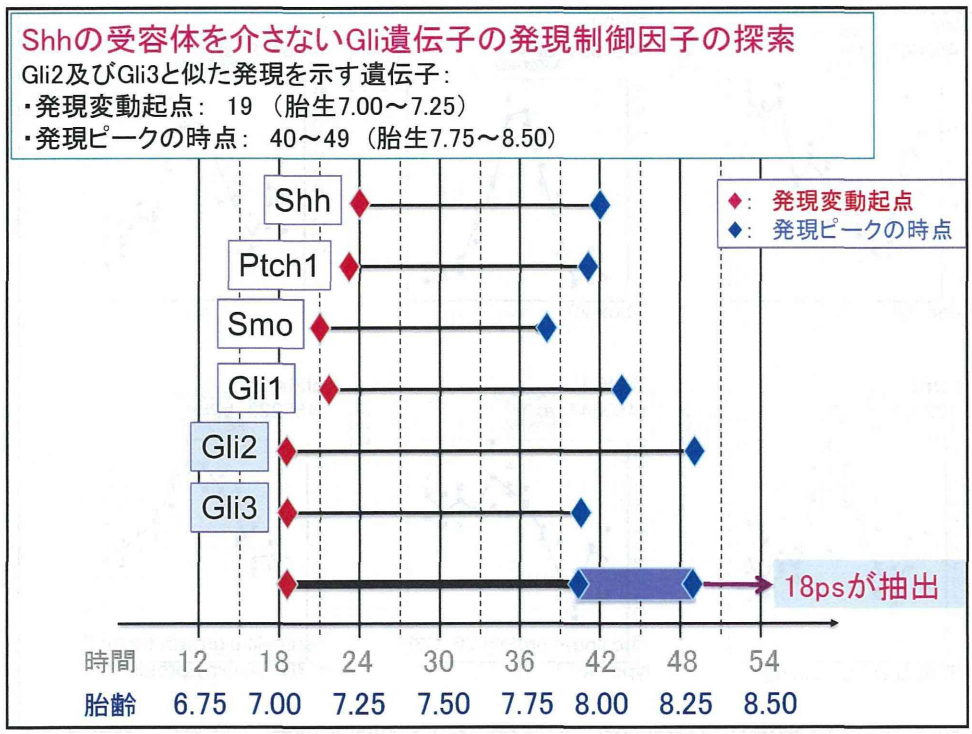
→ 発現変動起点が19のGli遺伝子
発現制御遺伝子が想定



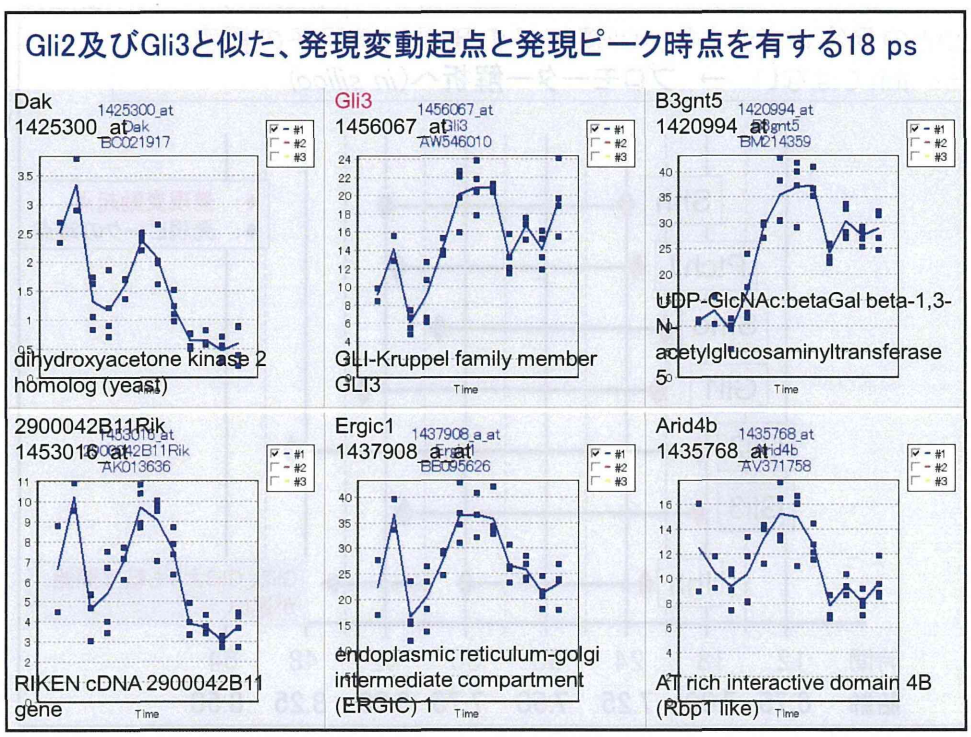
Zhang XM et al, Cell 106(2): 781-792, 2001.

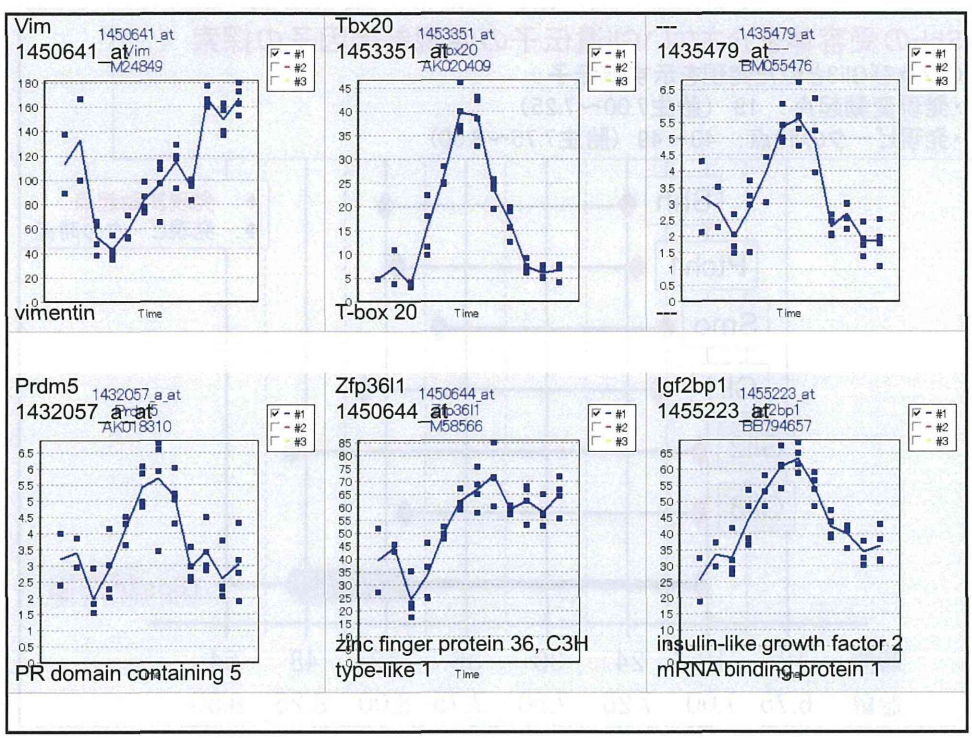


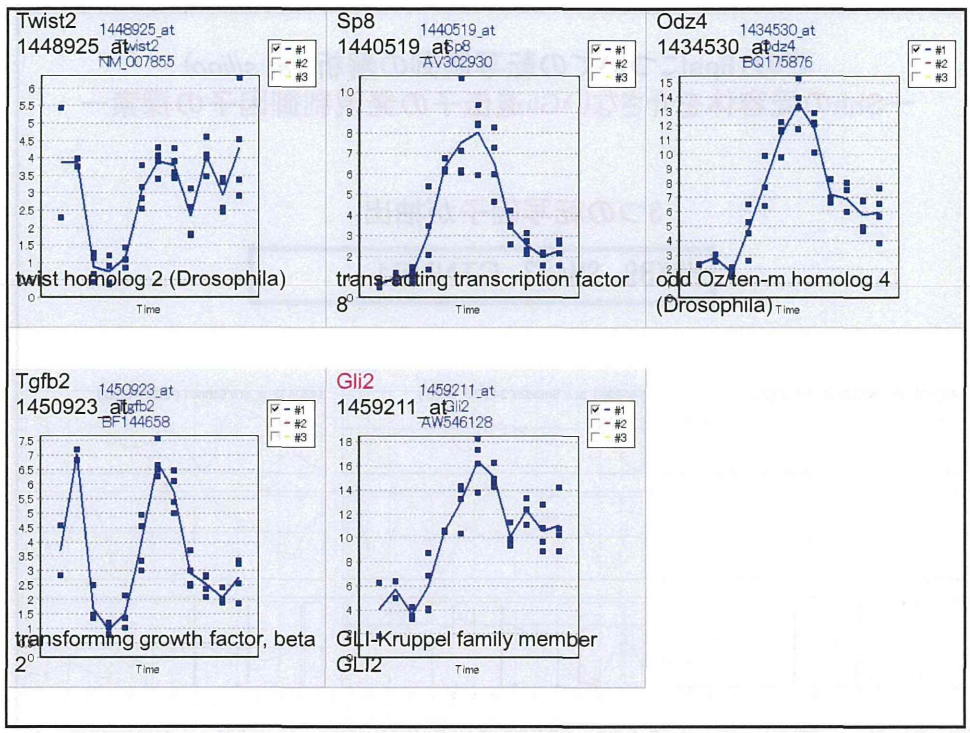

Shhの受容体を介さないGli遺伝子の発現制御因子の探索
→ Ihhではない → プロモーター解析へ(in silico)
◆: 発現変動起点
◆: 発現ピークの時点
Shh
Ptch1
Smo
Gli1
Gli2
Gli3
Ihh
Gli2、Gli3よりも転写開始が遅い
時間 12 18 24 30 36 42 48 54
胎齢 6.75 7.00 7.25 7.50 7.75 8.00 8.25 8.50


Shhの受容体を介さないGli遺伝子の発現制御因子の探索
Gli2及びGli3と似た発現を示す遺伝子:
・発現変動起点: 19 (胎生7.00～7.25)
・発現ピークの時点: 40～49 (胎生7.75～8.50)
◆: 発現変動起点
◆: 発現ピークの時点
Shh
Ptch1
Smo
Gli1
Gli2
Gli3
18psが抽出
時間 12 18 24 30 36 42 48 54
胎齢 6.75 7.00 7.25 7.50 7.75 8.00 8.25 8.50

Gli2及びGli3と似た、発現変動起点と発現ピーク時点を有する18 ps







この18psについての転写制御の解析（*in silico*）
ーShhの受容体を介さないGli遺伝子の発現制御因子の探索ー

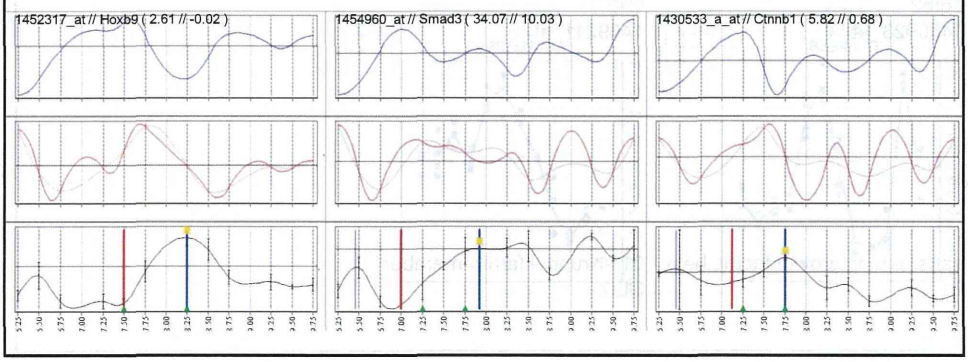
既存の発現制御データベースIngenuity Pathways Analysis (IPA)
(Ingenuity Systems Inc.)におけるUpstream Analysisを用いて検討

Upstream R	Fold Chang	Molecule T	Predicted A	Activation z	Notes	p-value of o	Target molecules in dataset
ZNF652		other				1.26E-05	TGFB2,VIM
IDH2		enzyme				4.68E-05	TGFB2,VIM
LAMC1		other				5.45E-05	TGFB2,VIM
POU5F1		transcriptio				6.79E-05	IGF2BP1,TGFB2,VIM
MUC4		growth fact				9.15E-05	TWIST2,VIM
HOXB9		transcriptio				1.02E-04	GLI3,TGFB2
sorafenib		chemical dı				1.51E-04	TWIST2,VIM
miR-141-3p		mature mic				2.25E-04	TGFB2,VIM
IDH1		enzyme				2.42E-04	TGFB2,VIM
MSX2		transcriptio				3.33E-04	TGFB2,VIM
SMAD3		transcriptio				4.39E-04	GLI2,TGFB2,VIM
Laminin		complex				4.62E-04	TGFB2,VIM
SNAI2		transcriptio				5.09E-04	TWIST2,VIM
CDH1		other				6.65E-04	BMP7,VIM
EPHB4		kinase				6.93E-04	BMP7,TGFB2
estrogen re		group				7.18E-04	BMP7,TGFB2,VIM
crizotinib		chemical dı				7.99E-04	VIM
SPRED2		cytokine				7.99E-04	VIM
FAF1		other				7.99E-04	VIM
CTNNB1		transcriptio				8.24E-04	BMP7,GLI2,IGF2BP1,TBX20

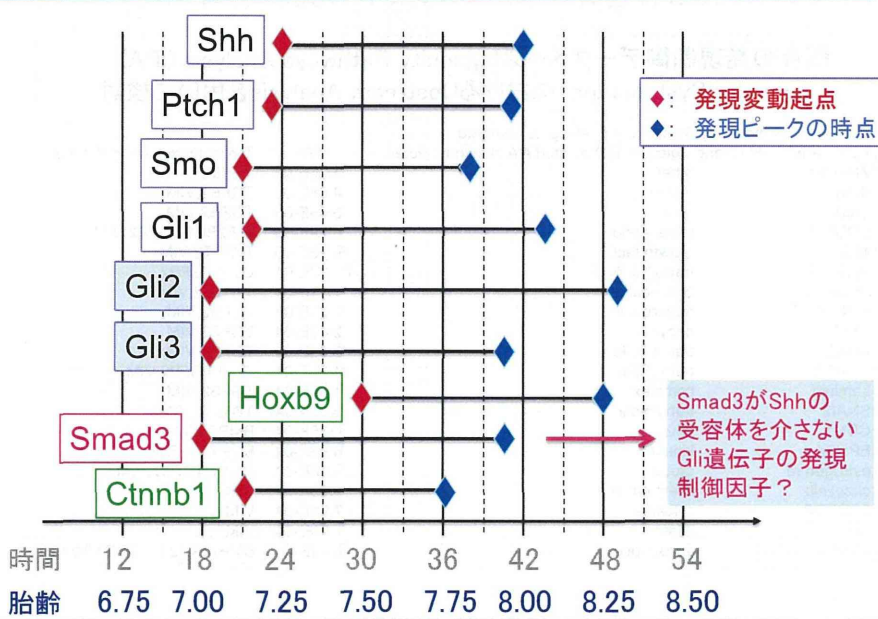
この18psについての転写制御の解析（*in silico*）
―Shhの受容体を介さないGli遺伝子の発現制御因子の探索―

3つの転写因子が抽出

HOXB9、SMAD3、CTNNB1



Shhの受容体を介さないGli遺伝子の発現制御因子の探索(*in silico*)
→ HOXB9、SMAD3、CTNNB1 が候補



Shhの受容体を介さないGli遺伝子の発現制御因子の探索(*in silico*)

→ SMAD3　：胎児期での検討ではないが、発現制御を示唆する報告有り

Induction of Sonic Hedgehog Mediators by Transforming Growth Factor-β: Smad3-Dependent Activation of *Gli2* and *Gli1* Expression *In vitro* and *In vivo*

Sylviane Dennler,[1] Jocelyne André,[1] Ismini Alexaki,[1] Allen Li,[2] Thierry Magnaldo,[3] Peter ten Dijke,[4] Xiao-Jing Wang,[2] Franck Verrecchia,[1] and Alain Mauviel[1]

[1]Institut National de la Santé et de la Recherche Médicale U697, Paris, France; [2]Department of Otolaryngology, Oregon Health and Science University, Portland, Oregon; [3]Centre National de la Recherche Scientifique UPR 2169, Institut Gustave-Roussy, Villejuif, France; and [4]Department of Molecular Cell Biology, Leiden University Medical Center, Leiden, the Netherlands

Abstract

Hedgehog (Hh) and transforming growth factor-β (TGF-β) family members are involved in numerous overlapping processes during embryonic development, hair cycle, and cancer. Herein, we show that TGF-β induces the expression of the Hh signaling molecules *Gli1* and *Gli2* in various human cell types, including normal fibroblasts and keratinocytes, as well as various cancer cell lines. *Gli2* induction by TGF-β is rapid, independent from Hh receptor signaling, and requires a functional Smad pathway. *Gli1* expression is subsequently activated in a Gli2-dependent manner. In transgenic mice overexpressing TGF-β1 in the skin, *Gli1* and *Gli2* expression is also elevated and depends on Smad3. In pancreatic adenocarcinoma cell lines resistant to Hh inhibition, pharmacologic blockade of TGF-β signaling leads to repression of cell proliferation accompanied with a reduction in *Gli2* expression. We thus identify TGF-β as a potent transcriptional inducer of Gli transcription factors. Targeting the cooperation of Hh and TGF-β signaling may provide new therapeutic opportunities for cancer treatment. [Cancer Res 2007;67(14):6981–6]

[Cell Cycle 6:24, 2456-2463, 15 October 2007]; ©2007 Landes Bioscience

Perspective

Non-Canonical Activation of GLI Transcription Factors

Implications for Targeted Anti-Cancer Therapy

Matthias Lauth
Rune Toftgård*

Karolinska Institutet, Center for Biosciences, Department of Biosciences and Nutrition, Novum Research Park, Huddinge, Sweden

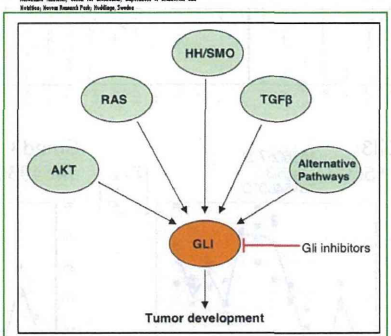
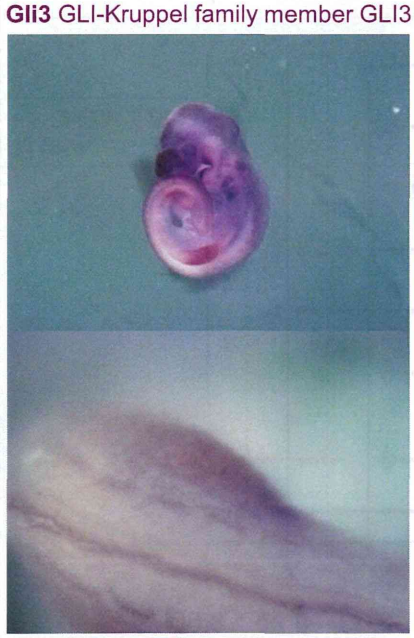


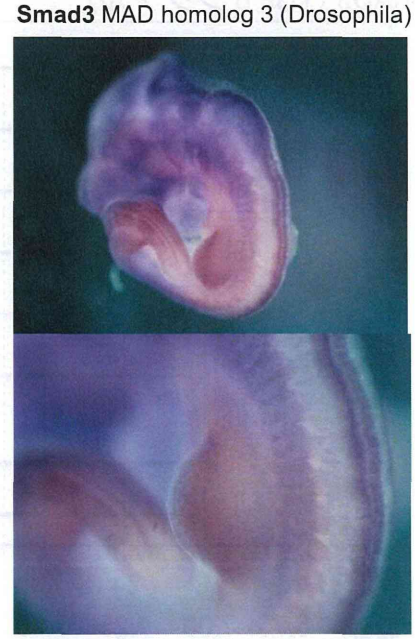
Figure 2. Many signaling pathways converge on GLI. The HH/SMO pathway (canonical HH signaling) might be just one of many ways to activate GLI transcription. All non-canonical activation mechanisms identified so far interact with the HH pathway downstream of SMO. Hence, a GLI inhibitor is well suited for targeted therapeutic approaches in numerous malignancies characterized by elevated GLI activity. 'Alternative pathways' indicates signaling pathways, yet to be identified, interacting with HH/GLI.

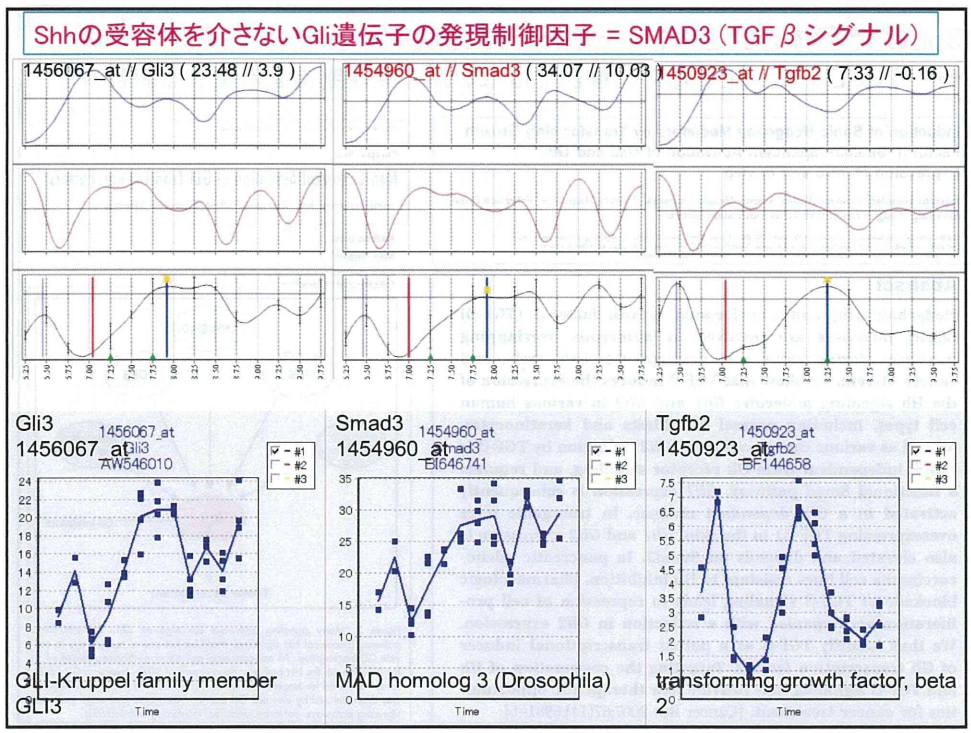
Emage検索によるISHデータ

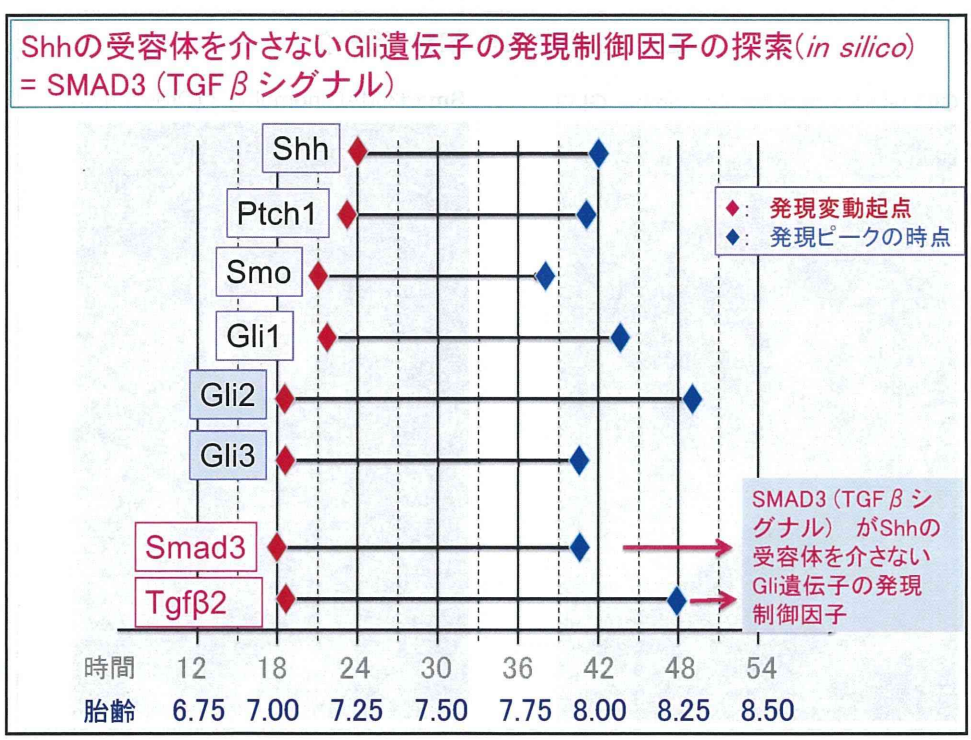
Gli3 GLI-Kruppel family member GLI3



Smad3 MAD homolog 3 (Drosophila)




Shhの受容体を介さないGli遺伝子の発現制御因子 = SMAD3 (TGFβシグナル)
1456067_at // Gli3 (23.48 // 3.9)
1454960_at // Smad3 (34.07 // 10.03)
1450923_at // Tgfb2 (7.33 // -0.16)
Gli3
1456067_at
AW546010
GLI-Kruppel family member GLI3
Smad3
1454960_at
BI646741
MAD homolog 3 (Drosophila)
Tgfb2
1450923_at
BF144658
transforming growth factor, beta 2
Time


Shhの受容体を介さないGli遺伝子の発現制御因子の探索(in silico)
= SMAD3 (TGFβシグナル)
Shh
Ptch1
Smo
Gli1
Gli2
Gli3
Smad3
Tgfβ2
◆: 発現変動起点
◆: 発現ピークの時点
SMAD3 (TGFβシグナル) がShhの受容体を介さないGli遺伝子の発現制御因子
時間 12 18 24 30 36 42 48 54
胎齢 6.75 7.00 7.25 7.50 7.75 8.00 8.25 8.50