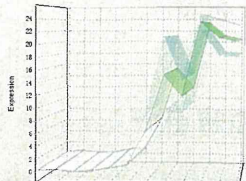


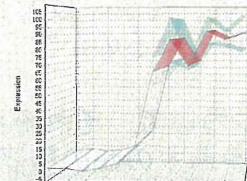
胎生9.25日

Myl2
1448394_at



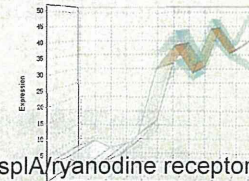
myosin, light polypeptide 2, regulatory, cardiac, slow

Crabp1
1448326_a_at



cellular retinoic acid binding protein I

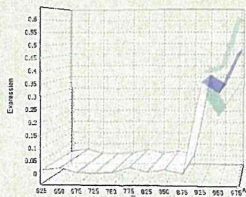
Spsb4
1451418_a_at



splA/ryanodine receptor domain and SOCS box containing 4

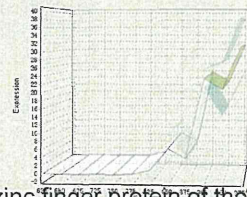
胎生9.75日

Dlx1
1449470_at



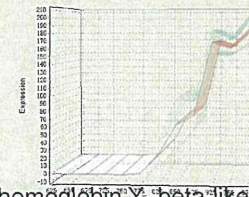
distal-less homeobox 1

Zic1
1439627_at



zinc finger protein of the cerebellum 1

Hbb-y
1450621_a_at



hemoglobin Y₁-beta-like embryonic chain

周波数解析は周波数に着目した解析であり、直接、遺伝子発現の経時パターンを反映したものではない

Q1: どの程度、発現パターンを反映しているのか?

→

- ・ Shh遺伝子が含まれる、発現の最大ピークが胎生8.50日である234 psについての検討
- ・ 各時点での代表例について検討(各3遺伝子)

・ Shh遺伝子が含まれる発現の最大ピークを含む時点・胎生8.5日以降の時点では発現パターンが比較的似ているものが抽出されてきたが、それ以前の時点の場合、発現パターンがかなり異なるものが抽出されてきた。

目視による確認にかわる、自動認識手法*の導入を検討する。

*: 発現パターンを標準化しピーク特性を評価する

Q2: Shh関連遺伝子は、発現の最大ピークを示すどの時点に分類されているのか？

→Shh関連遺伝子について、それぞれの時点で発現の最大ピークを示すのかの確認

Shh、Ptch1、Smo、Gli2、Gli3

Shh遺伝子を基としフーリエ変換して得られた波長分布について
ピアソン相関解析の結果得られた(相関係数>0.90)

7,039 psについて、
RSortで経時的に並びかえ(3.5日間のフォーマットで)、発現の最大ピークを示す
各時点毎に抽出(目視の検討はなし)

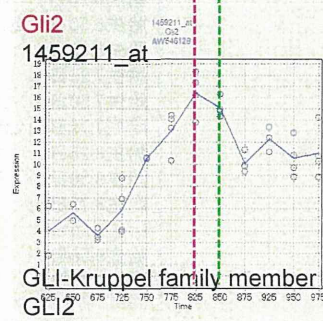
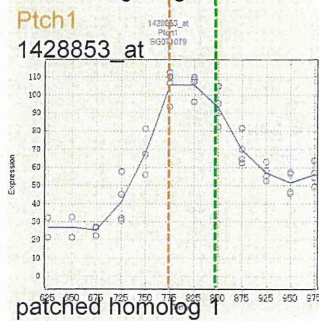
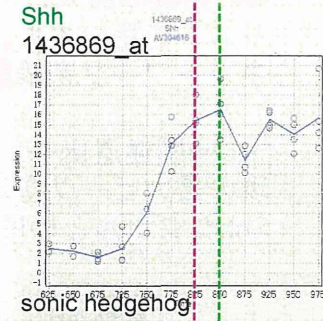
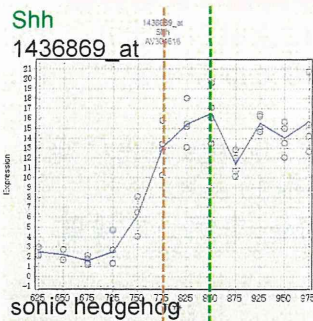
Shh遺伝子: 胎生8.5日に発現ピークを持つ遺伝子として抽出されてきた

結果 ← 7,039 ps

胎生6.25日:	460 ps	
胎生6.50日:	2,714 ps	
胎生6.75日:	55 ps	
胎生7.25日:	13 ps	
胎生7.50日:	235 ps	
胎生7.75日:	2,813 ps	Ptch1、Smo
胎生8.25日:	249 ps	Gli2、Gli3
胎生8.50日:	234 ps	Shh
胎生8.75日:	0 ps	
胎生9.25日:	80 ps	
胎生9.50日:	5 ps	
胎生9.75日:	181 ps	

Setting:
Explore Direction: Peak>>>Valley
Posi,Intermed,Nega:全てcheck

Shhシグナル関連遺伝子の発現変動



Q2: Shh関連遺伝子は、発現の最大ピークを示すどの時点に分類されているのか？

→Shh関連遺伝子について、それぞれどの時点で発現の最大ピークを示すのかの確認

Shh、Ptch1、Smo、Gli2、Gli3

発現の最大ピークだけを指標に分類し、解析していく手法では不十分

→発現の立ち上がりの勾配（微分値等）を考慮すれば解決する可能性が高い

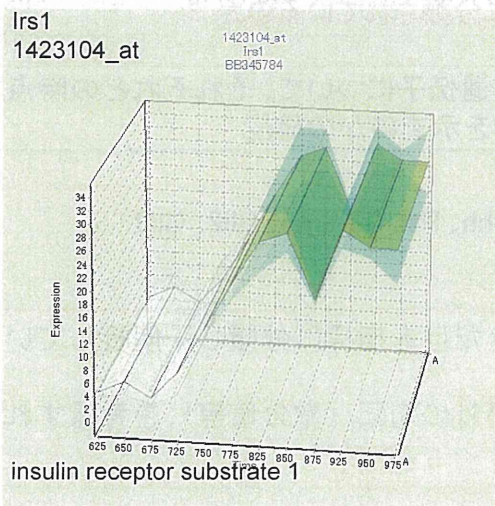
→ 今後の課題

Q3: 発現の最大ピークを示す時点による分類リストを用いても、本手法によりシグナルネットワークを検索できるのではないか？

→Shh関連遺伝子が含まれない胎生9.25日に発現の最大ピークを示す遺伝子(80 ps)について、Shhシグナルとの関連をPubMedを用いて検索

胎生6.25日:	460 ps	
胎生6.50日:	2,714 ps	
胎生6.75日:	55 ps	
胎生7.25日:	13 ps	
胎生7.50日:	235 ps	
胎生7.75日:	2,813 ps	Ptch1, Smo
胎生8.25日:	249 ps	Gli2, Gli3
胎生8.50日:	234 ps	Shh
胎生8.75日:	0 ps	
胎生9.25日:	80 ps	
胎生9.50日:	5 ps	
胎生9.75日:	181 ps	

insulin receptor substrate 1



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

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-L¹, Dane Ohlsson Campbell¹ 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.

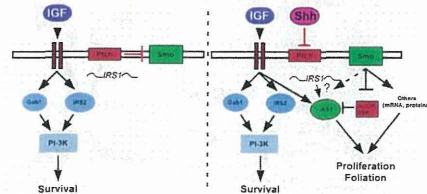
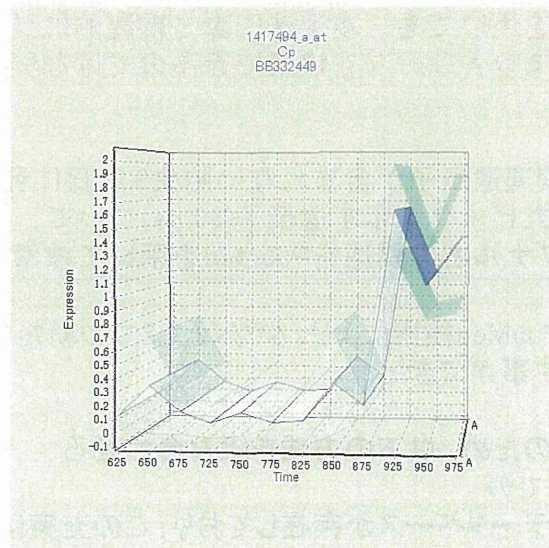


Fig. 7. Model suggesting how SHH mediates IRS1 expression during CGNP proliferation. In the absence of SHH, IGF signaling sends survival cues through IRS2 and/or GAB1 and PI-3K signaling (left panel). IRS1 mRNA is present. In the presence of SHH, IRS1 is upregulated by a mechanism that may involve both 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.

ceruloplasmin



胎生6.25-9.75日で検討したwhole mount ISHデータが見いだせなかった

Identification of Sonic Hedgehog-Responsive Genes Using cDNA Microarray

Sonic hedgehog (Shh) is a secreted signaling protein that plays important roles in a variety of developmental processes and also in pathogenesis of some human cancers and congenital diseases. Molecules that function downstream of Shh, however, still remain elusive. Here we searched for Shh-responsive genes by using an in-house cDNA microarray. Two genes were newly identified to be Shh responsive in neuroepithelial cell line MNS-70: the metal-binding protein *Ceruloplasmin* (*Cp*) and the serine protease inhibitor *inter- α -trypsin inhibitor heavy chain H3* (*ITIH3*). In MNS-70 cells, expression of *ITIH3* was regulated by Gli zinc-finger transcription factors downstream of Shh, whereas *Cp* appeared to be regulated by Gli-independent pathways. *Cp* mRNA was detected in the developing mouse brain, where its expression domain was closely adjacent to that of *Shh*. These results demonstrate that microarray technology provides a useful tool for studying expression of developmentally regulated genes. © 2001 Elsevier Science

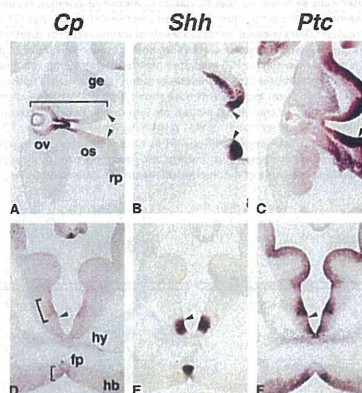


FIG. 3. Comparison of the expression pattern of *Cp* with those of *Shh* and *Ptc* in the developing brain. Transverse sections of the E13 mouse brain was subjected to *in situ* hybridization with anti-sense probes for *Cp* (A, D), *Shh* (B, E) and *Ptc* (C, F). A-C and D-F show adjacent sections. Arrowheads indicate the positions of the sharp expression boundaries of *Shh*. Brackets indicate the regions where *Cp* mRNA is detected. Note that the expression of *Cp* in the mid-stalk (A), hypothalamus, and ventral region of the hindbrain (D) flanks that of *Shh*, whereas *Ptc* mRNA is detectable in much broader regions. Abbreviations used: fp, floor plate; ge, ganglionic eminence; hb, hindbrain; hy, hypothalamus; os, optic stalk; ov, optic vesicle; rp, Rathke's pouch.

Q3: 発現の最大ピークを示す時点による分類リストを用いても、本手法により網羅的にシグナルネットワークを検索できるのではないか？

→Shh関連遺伝子が含まれない胎生9.25日に発現の最大ピークを示す遺伝子(80 ps)について、Shhシグナルとの関連をPubMedを用いて検索

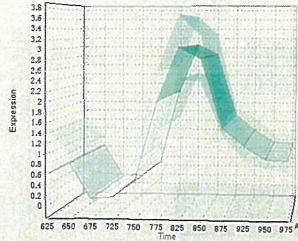
80psについてのPubMed検索により、少なくとも2つの新たなShh関連遺伝子を見いだす事ができた。

抽出精度の向上のため、以下の方法を組み合わせる

- wholemount ISHでの
→胚でのISHのデータベースが存在しており、この充実に期待
- *in silico*のプロモーター解析

胎生8.25日の時点でもShh関連遺伝子を見いだすことができた

LOC100044104 /// Tbx1
1425779_a_at



T-box 1 /// similar to T-box 1

Developmental Biology 24, 12-13 (2001)

doi:10.1006/dbio.2001.0285, available online at www.idealibrary.com - **IDEAL**

Tbx1, a DiGeorge Syndrome Candidate Gene, Is Regulated by Sonic Hedgehog during Pharyngeal Arch Development

Vidu Garg,* Chihiro Yamagishi,*† Tonghuan Hu,*†
Irfan S. Kathiriyai,*† Hiroyuki Yamagishi,*† and Deepak Srivastava*†
*Department of Pediatrics, and †Department of Molecular Biology, University of Texas
Southwestern Medical Center at Dallas, 6505 Harry Hines Boulevard,
Rm. NAB 124, Dallas, Texas 75390-9148

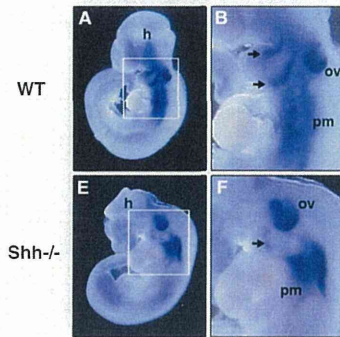


FIG. 4. *Tbx1* expression is downregulated in the pharyngeal arches of *Shh*-null mouse embryos. *Tbx1* expression in E10.5 embryos by whole-mount (A, B, E, F) and radiolabeled *in situ* hybridization of coronal sections (C, D, G, H). *Tbx1* expression in the mesodermal core of the pharyngeal arches (arrows) in *Shh*-null embryos (E, F) was decreased compared to wild-type embryos (A, B), while expression was

胚でのISHの公開データベース

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例: Shh

Gene/Protein	Theler Stage	Structure	Find Similar	3D Expression	2D Expression	Data Image
Shh (1)	TS12 (12)	<input type="checkbox"/> Show all foregut diverticulum hindgut diverticulum endoderm notochord	<input type="checkbox"/> Show all hindgut diverticulum endoderm notochord	<input type="checkbox"/> Show all diencephalon floorplate embryo future midbrain floorplate	<input type="checkbox"/> Show all 1st arch mandibular part diencephalon floorplate	(44)

周波数解析は周波数に着目した解析であり、直接、遺伝子発現の経時パターンを反映したものではない
Q1: どの程度、発現パターンを反映しているのか？

Shh遺伝子が含まれる発現の最大ピークを含む時点・胎生8.5日では、周波数に着目した解析であっても、遺伝子の発現パターンを反映したものが抽出できている

・Shh遺伝子が含まれる発現の最大ピークを含む時点・胎生8.5日以降の時点では発現パターンが比較的似ているものが抽出されてきたが、それ以前の時点の場合、発現パターンがかなり異なるものが抽出されてきた。

目視による確認にかわる、自動認識手法*の導入を検討する。*: 発現パターンを標準化しピーク特性を評価する

Q2: Shh関連遺伝子は、発現の最大ピークを示すどの時点に分類されているのか？

発現の最大ピークだけを指標に分類し、解析していく手法では不十分

→発現の立ち上がりの勾配（微分値等）を加味する必要がある

→ 今後の課題

Q3: 発現の最大ピークを示す時点による分類リストを用いても、本手法により網羅的にシグナルネットワークを検索できるのではないか？

80psについてのPubMed検索により、少なくとも2つの新たなShh関連遺伝子を見いだす事ができた。

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・wholmount ISHでの

→胚でのISHのデータベースが存在しており、この充実に期待

・*in silico*のプロモーター解析

Nkx2.5シグナリング（心筋前駆細胞マーカー、心臓発生過程）
についての検討

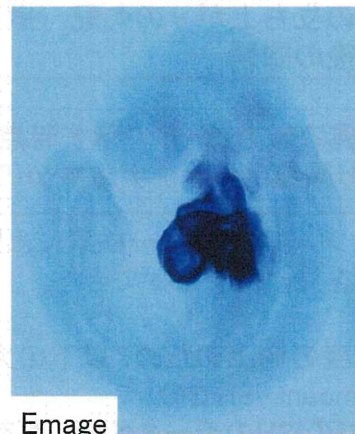
マウス胚におけるNkx2-5の発現

胎生8.75日



Harvey et al, 1996

胎生9.5日



Emage

初期心臓前駆細胞で発現するマーカー: Mesp1、Flk1 (Kdr)

↓
心筋前駆細胞マーカー: Nkx2.5 (Nkx2-5)

↓
心筋マーカー: Cardiac actin (Actc1), Troponin T2 (Tnnt2)

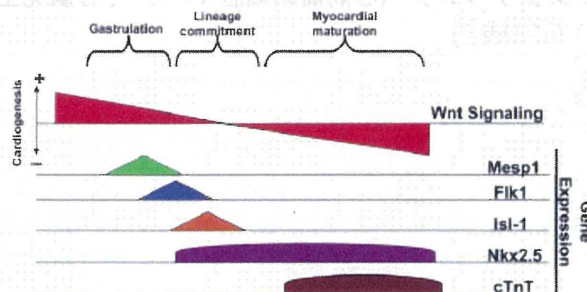


Figure 1. The Biphasic Effect of Wnt Signaling in Cardiac Mesoderm Induction and Myocardial Differentiation

The role of Wnt signaling in cardiovascular lineage specification and differentiation is depicted in the context of the expression of mesoderm and cardiac transcription factors during embryogenesis. cTnT, cardiac troponin T.

Wu SM, Mesp1 at the heart of mesoderm lineage specification. Cell Stem Cell 2008 3: 1-2, 2008.