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－在留中国人研究者研究助成－

２００４年　３　月１０日

財団法人　日中医学協会
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１．研究テーマ

心臓の形態形成におけるセマフォリン分子 Sema6D の役割

２．本年度の研究業績

(1) 学会・研究会等における発表　(有)・無　（学会名・演題）

第 68 回日本循環器学会総会

Novel Transmembrane Type Class VI Semaphorin, Sema6D Regulates Cardiac Morphogenesis Through PlexinA1

(2) 学会誌等に発表した論文　有・(無)　（雑誌名・論文名）

3．今後の研究計画

セマフォリン分子 Sema6D の欠損マウスを作成して、哺乳動物で Sema6D は心臓の形態形成に関わることを検討します。

4．指導責任者の意見

張弘は北京医科大学を１９９３年卒業し、６年間北京医科大学附属病院において循環専門医として診療と臨床研究に取り組んできた。１９９９年より大阪大学医学部多田教授として心筋細胞の再生を目標に、心臓の発生に関与する因子の同定を開始した。増殖因子の機能解析のために自らニワトリ胎児胚を用いた実験系を構築した。最近は、神経発生に重要なセマフォリン分子に着目した研究により、分子群の新しいセマ６Ｄが心臓の形態形成に冠よすることを発見した。これらの成果は生物現象を蛋白質間相互作用や細胞(細胞集団)レベルに再構成して追求せんとする彼女独特の研究姿勢に帰するところが大きいといえます。彼女の研究能力はポストゲノム、再生医学へと展開しつつある２１世紀に向けての新しい循環器学の発展に資するものと期待されます。

指導責任者氏名 堀 正二 ㊞

5．研 究 報 告 書

別紙「研究報告書の作成について」に倣い、指定の用紙で作成して下さい。

研究発表または研究状況を記録した写真を添付して下さい。

※研究成果を発表する場合は、発表原稿・抄録集等も添付して下さい。

※発表に当っては、*日中医学協会助成金による*旨を明記して下さい。

—日中医学協会助成事業—

心臓の形態形成におけるセマフォリン分子 Sema6D の役割

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要旨

Semaphorins, originally identified as axon guidance factors in the nervous system, play integral roles in organogenesis. Here, we demonstrate a critical involvement of Sema6D in cardiac morphogenesis. Ectopic expression of Sema6D or RNA interference against Sema6D induces expansion or narrowing of the ventricular chamber, respectively, during chick embryonic development. Sema6D also exerts region-specific activities on cardiac explants: a migration-promoting activity on outgrowing cells from the conotruncal segment and a migration-inhibitory activity on those from the ventricle. Plexin-A1 mediates these activities as the major Sema6D-binding receptor. Plexin-A1 forms a receptor complex with vascular endothelial growth factor receptor type 2 in the conotruncal segment or with Off-track in the ventricle segment; these complexes are responsible for the effects of Sema6D on the respective regions. Thus, the differential association of Plexin-A1 with additional receptor components entitles Sema6D to exert distinct biological activities at adjacent regions. This is crucial for complex cardiac morphogenesis.

Key Words　Sema6D, plexinA1, OTK, VEGFR2, Cardiac morphogenesis

緒言

The semaphorin family of proteins is characterized by a phylogenetically conserved "sema domain" in the extracellular region. Based on additional structural features, such as the presence or absence of transmembrane domains, Ig-like domains, thrombospondin repeats, and glycophosphatidylinositol linkage sites, the family has been subdivided into eight groups, which also include virally derived proteins. Semaphorins were originally identified as axon guidance factor in the nervous system, including fasciculation, axon branching and target selection. Increasing evidence indicates that semaphorines play developmental and regulatory roles in organogenesis outside of the nervous system, such as angiogenesis, tumor growth and metastasis, and immune response. In this study, we isolated a class VI semaphorin,

Sema6D, from the mouse heart and assessed the role of Sema6D in organogenesis, utilizing the whole chick embryo culture system. Ectopic expression of Sema6D as well as RNA interference (RNAi) against Sema6D induced malformations in cardiac tube. Furthermore, Sema6D was found to participate in cardiac morphogenesis by exerting distinct biological activities through its receptor, Plexin-A1, that formed receptor complexes with OTK and vascular endothelial growth factor receptor type 2 (VEGFR2) in adjacent regions of the cardiac tube.

対象と方法:

1. *Chicken embryo cultures.* Fertilized chicken eggs were incubated at 38°C in high humidity. Embryos, staged according to the Hamburger and Hamilton method, were removed from eggs and placed ventral side up in culture dishes.
2. *Construction of cDNAs.* To isolate mouse cDNAs encoding semaphorin and plexins from total mouse heart cDNAs, we used a RT-PCR method using degenerative oligonucleotide primers.
3. *In situ hybridization.* Chick and mouse embryos at the desired stages were fixed and then hybridized with DIG-labeled antisense RNA probes. The cDNA sequences of cSema6D, cPlexin-A1, VMHC1, AMHC1, Sema6D, and Plexin-A1 were used to transcribe cRNAs for in situ hybridization probes.
4. *Gain of function assay of Seam6D.* control or sema6D-expressing cells were implanted into HH stage 4 chick embryos. These embryos were allowed to develop up to HHstage32. Ectopic expression of Sema6D resulted in abnormal cardiac tube formation, an expansion of the ventricle.
5. *Loss of function assay of Seam6D.* siRNAs for control, chick Sema6D and chick Plexin-A1 were chemically synthesized, then electroporated into precardiac mesoderm of HHstage7 chick embryo. Knockdown of the Sema6D and PlexinA1 reduced cardiac ventricle size, the narrowing of ventricle.
6. *Identification of receptor for Sema6D.* RT-PCR identified Plexin-A1, Plexin-A2, Plexin-A4, Plexin-B1 and NP1 mRNAs from mouse heart mRNAs. HEK293 cells were transfected with these DNAs and then incubated with alkaline phosphatase (AP) fused truncated Sema6D-Fc domain. AP-Sema6D-Fc specifically bound to Plexin-A1 indicating that Plexin-A1 is the major receptor for Sema6D.
7. *Collagen gel culture assay of cardiac explants.* The conotruncal (CT) segment and the ventricle of the heart from HH stage 14 chick embryos were dissected and placed onto collagen gels next to the transfectants that secreted sema6D. The outgrowths of the CT segment and the ventricle were measured. Sema6D enhanced the outgrowth from CT segment, but decreased that from ventricle segment..
8. *Identification of co-receptor for plexinA1.* RT-PCR indentified unique expression of VEGFR2 in CT segment, and Off-track in ventricle. RNAi against VEGFR2 and off-track inhibited sema6D effect on outgrowth from CT segment and ventricle explant, respectively.

結果:

1. Sema6D mRNA is expressed in the developing neural and cardiac tubes.
2. Ectopic expression of Sema6D and RNAi against cSema6D result in abnormal cardiac tube formation.
3. Sema6D induces distinct effects on the outgrowth from explants of differentregions of cardiac tube.
4. Plexin-A1 binds to Sema6D and its mRNA is expressed in the developing neural and cardiac tubes and mediates the effect of Sema6D on chick embryo.
5. Truncated Plexin-A1 and RNAi against cPlexin-A1 block the effect of Sema6D on cardiac explants.
6. Plexin-A1 interacts with OTK and VEGFR2.
7. Differential association of Plexin-A1 with OTK and VEGFR2 conveys the opposite effects of Sema6D on ventricle and CT segment.

考察:

Sema6D exerts region-specific activities on cardiac explants: a migration-promoting activity on outgrowing cells from the CT segment and a migration-inhibitory activity on those from the ventricle. Plexin-A1 mediates these activities as the major Sema6D-binding receptor. Plexin-A1 is associated with VEGFR2 in the CT segment and off-track in the ventricle explant. These receptor complexes determine the regional specific effect on cardiac morphogenesis by Sema6D. Thus, our findings present the clear evidence that the semaphorin system is critical in cardiac morphogenesis by regulating cell migration. Furthermore, the finding that the region-specific association of its canonical receptor with various receptor-type tyrosine kinases determines pleiotropic activities of Sema6D provides a new insight into the molecular basis of semaphorin signals.

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注：本研究は、2004年3月29日『第68回日本循環器学会総会』にて口演発表。

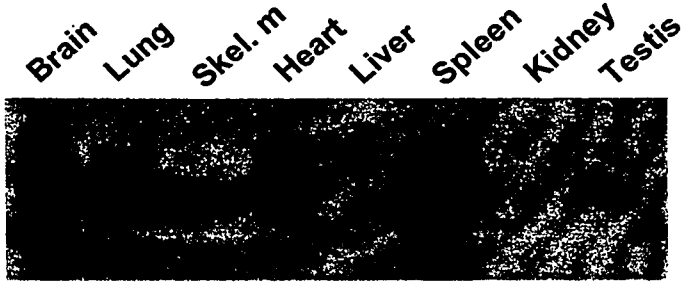
作成日：2004年3月10日

Cloning and expression of the novel class VI semaphorin, Sema6D

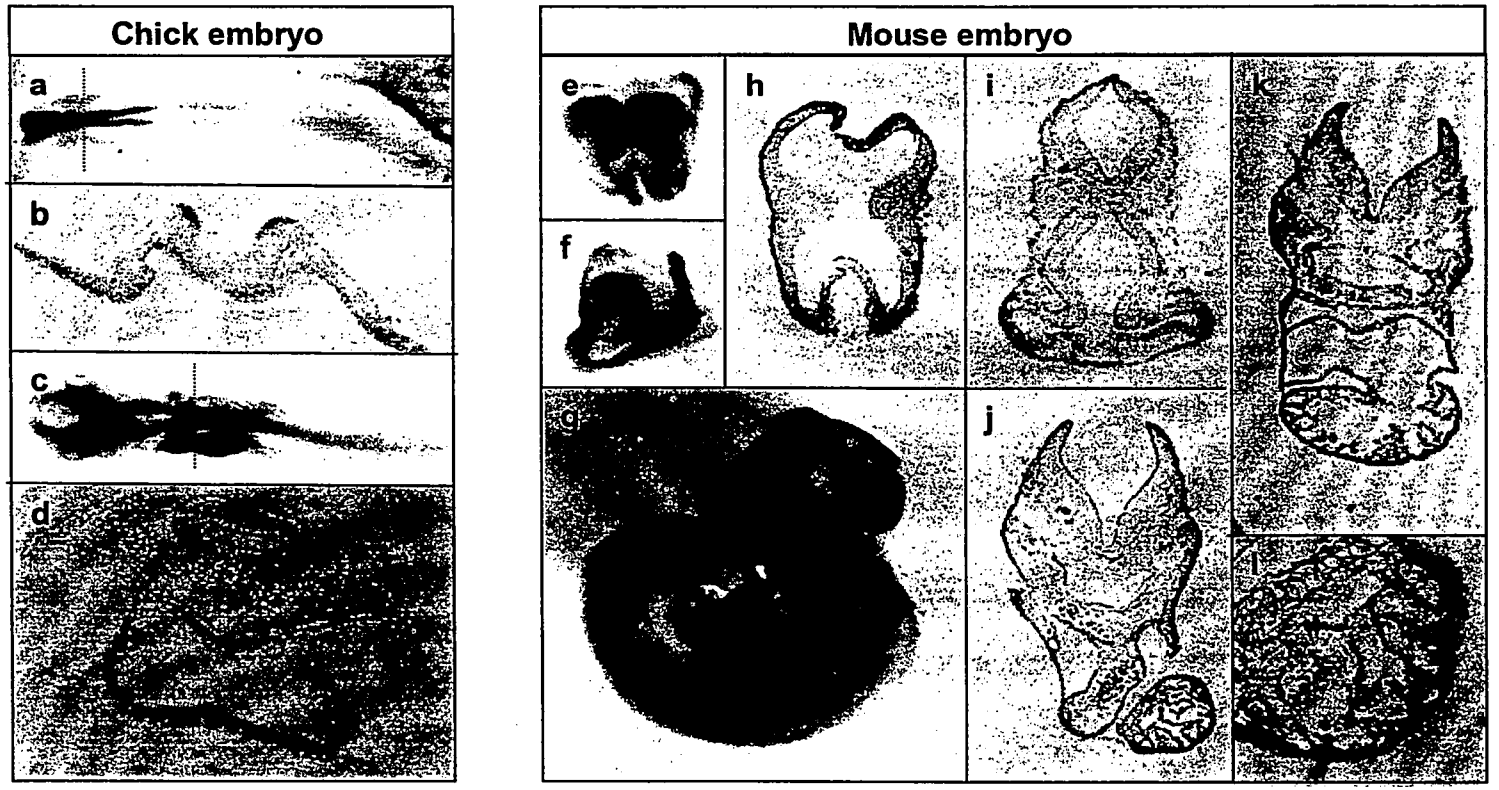
A *Amino acid comparison*

	Extra.	TM	Cyto.
M. Sema6D	100 %		100 %
H. Sema6D	98 %		95 %
C. Sema6D	96 %		95%
M. Sema6A1	52 %		34 %

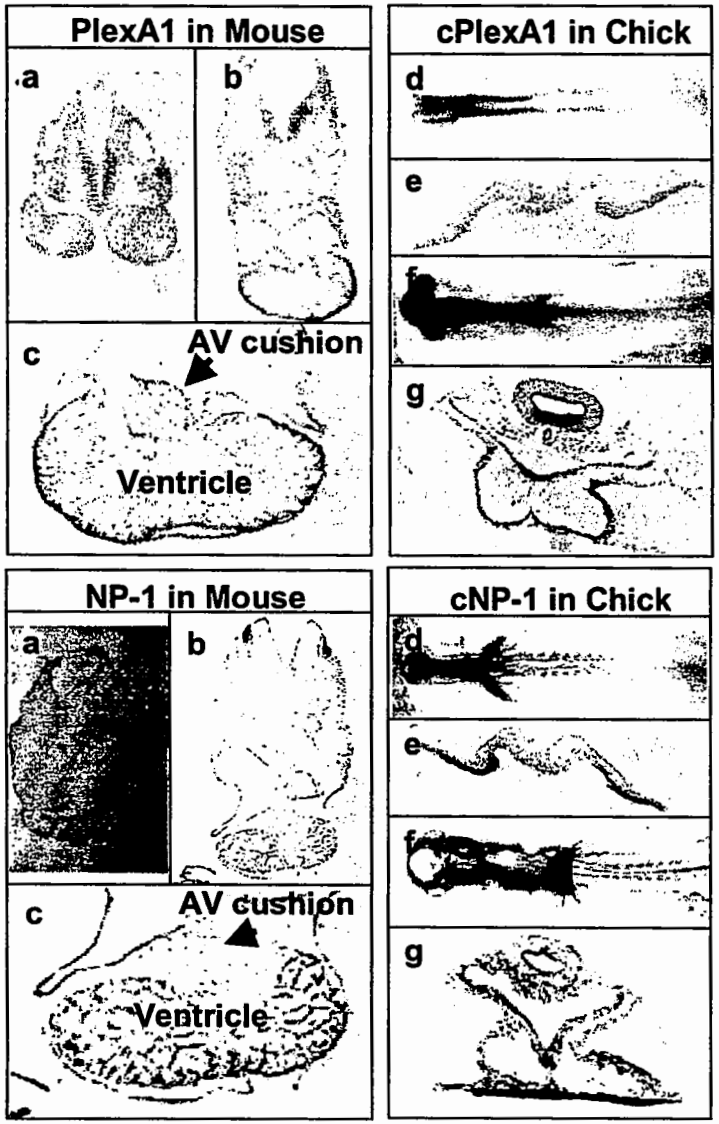
B *Sema6D mRNA in mouse adult tissues*



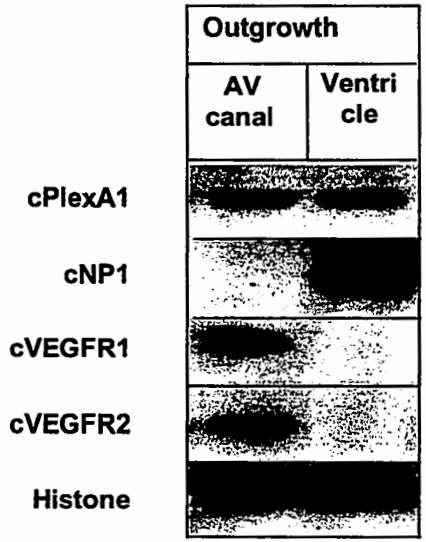
C *In situ* hybridization of Coll6D and mouse Sema6D mRNAs



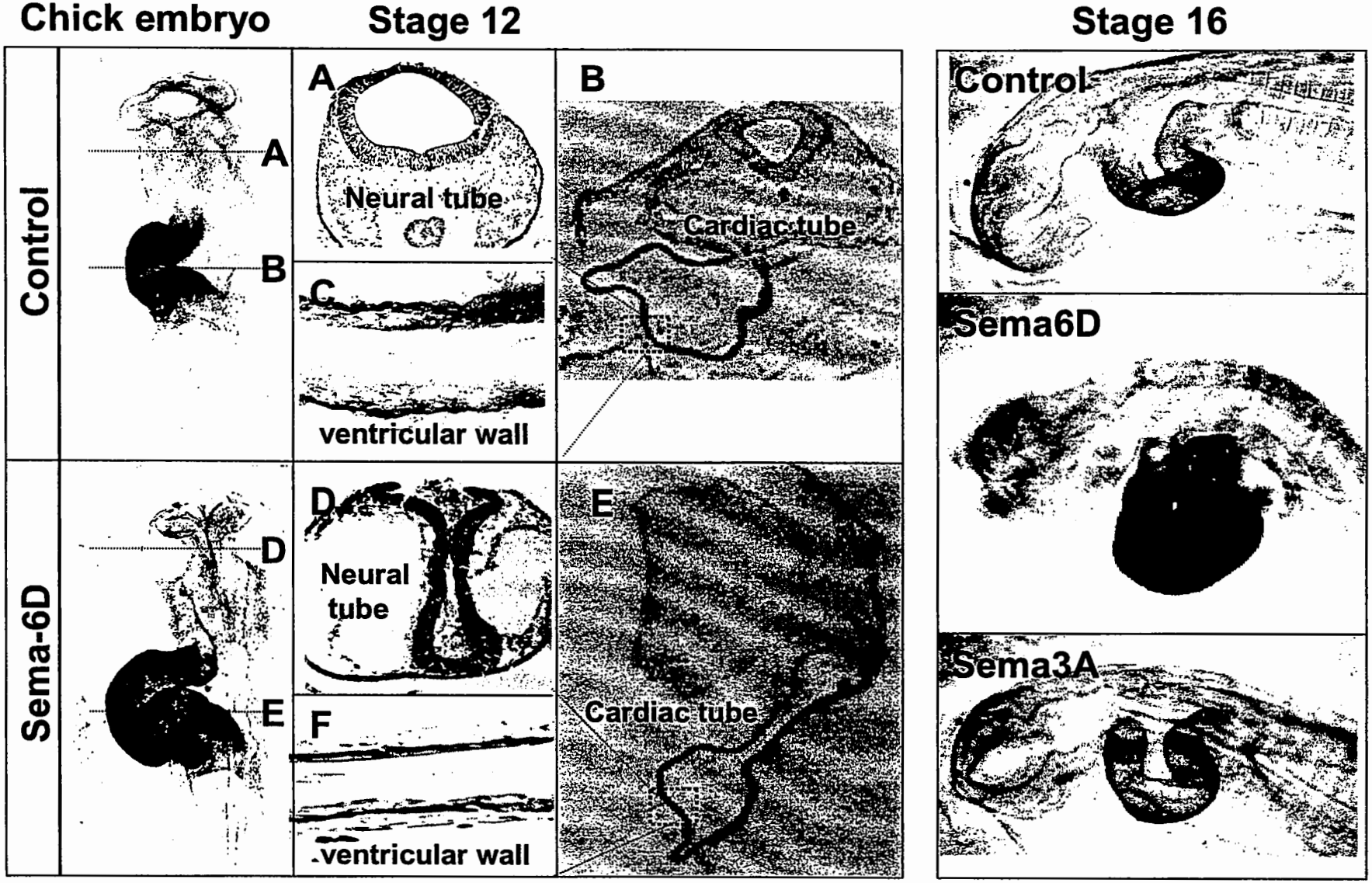
Expression of PlexinA1 and NP1



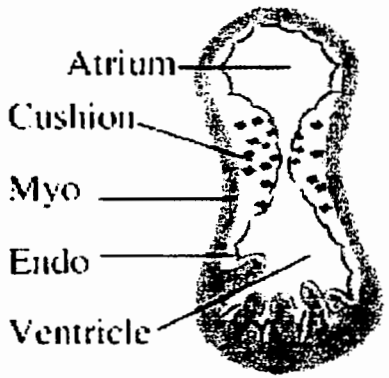
Expression of receptors in cardiac explants

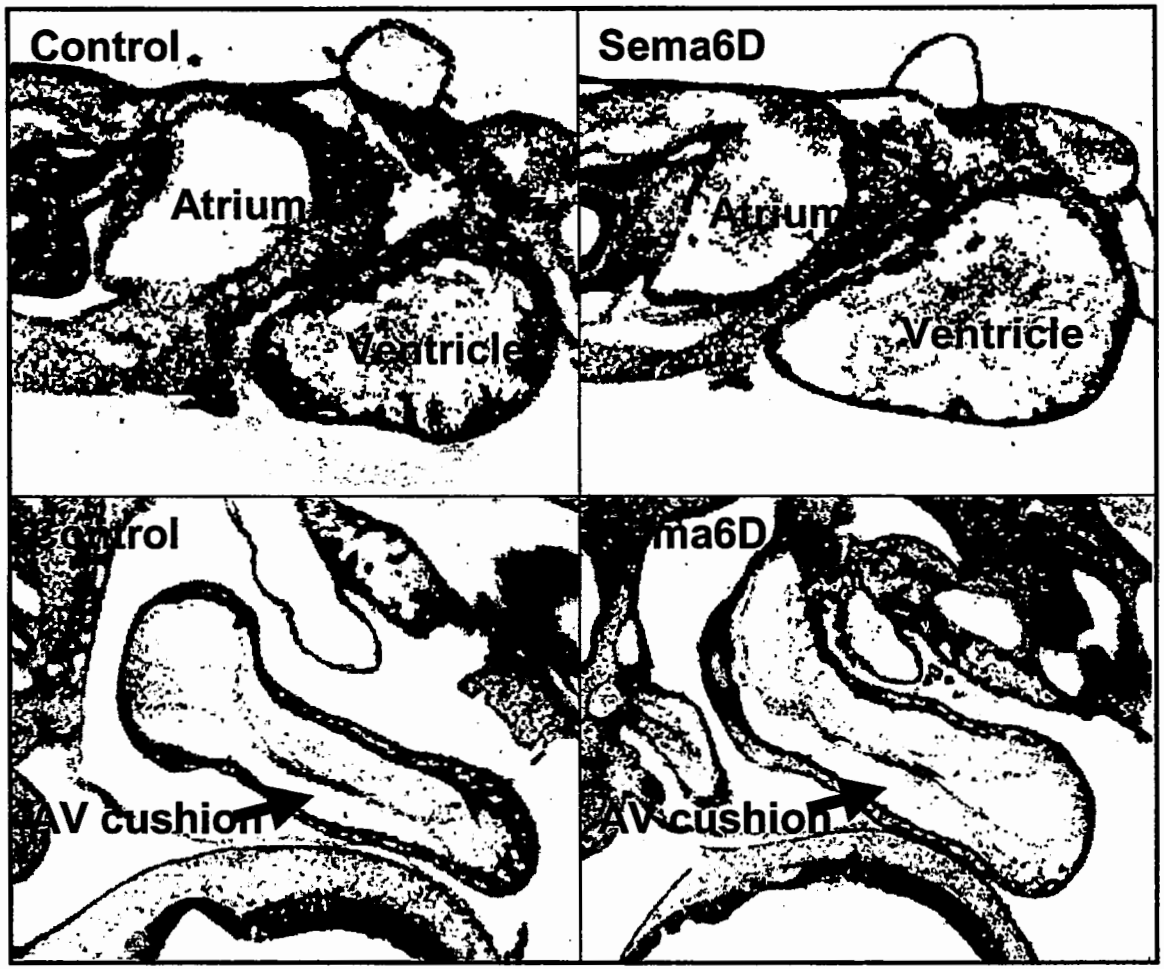


Ectopic expression of Sema6D suppresses neural tube formation and cardiogenesis

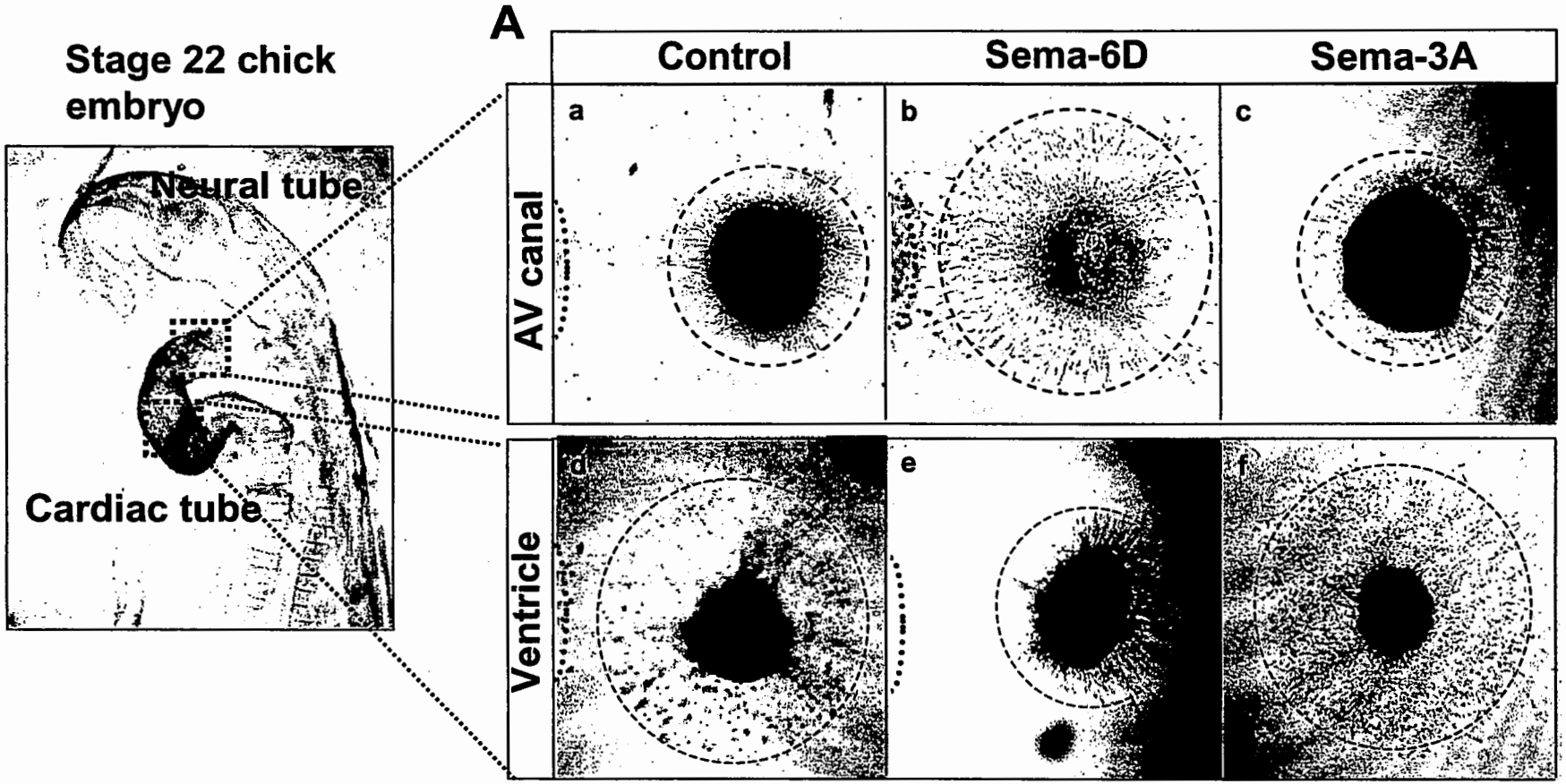


Sema6D suppresses myocardial trabeculation



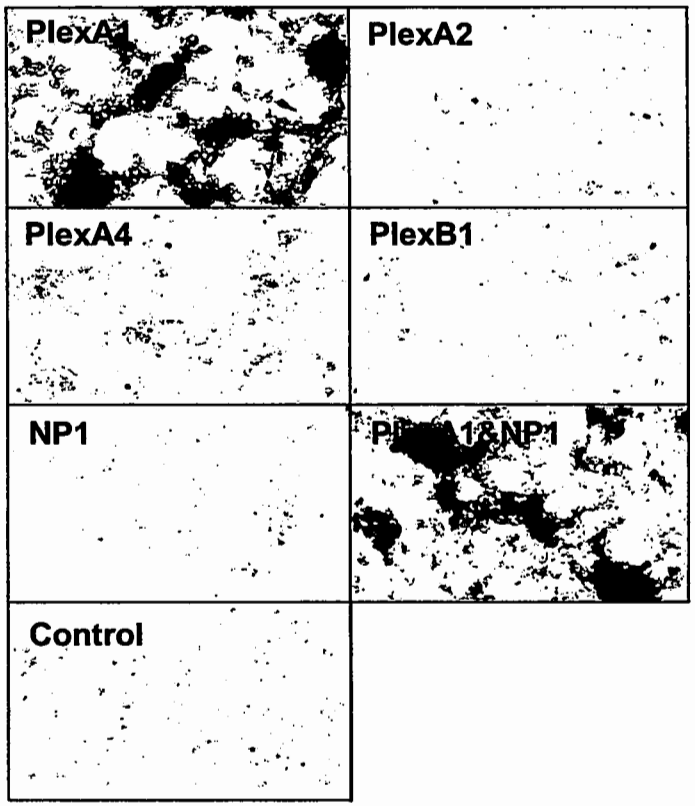


Sema6D regulates epithelial outgrowth from atrio-ventricular canal and ventricle

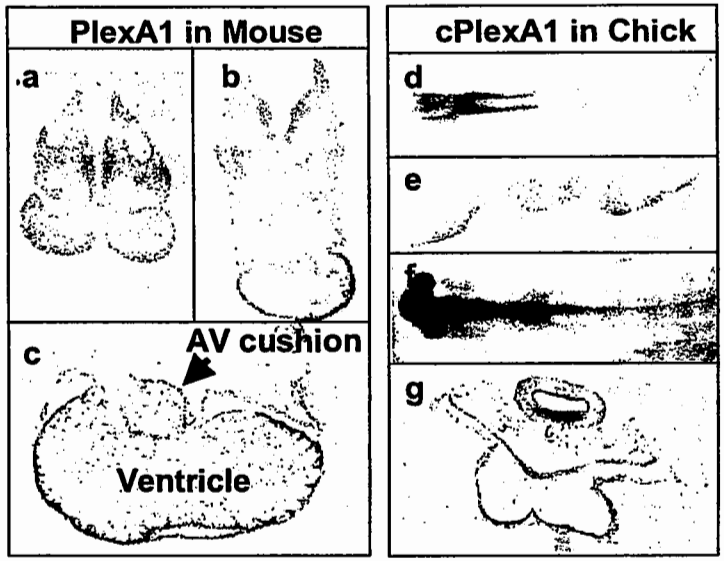


Sema6D binds to Plexin A1

A AP-Sema6D binds to PlexA1

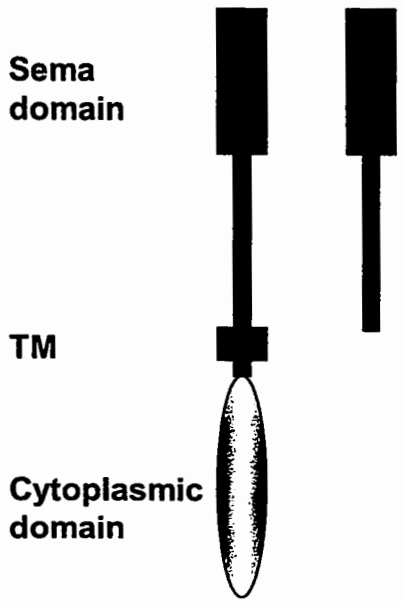


B Expression of PlexA1

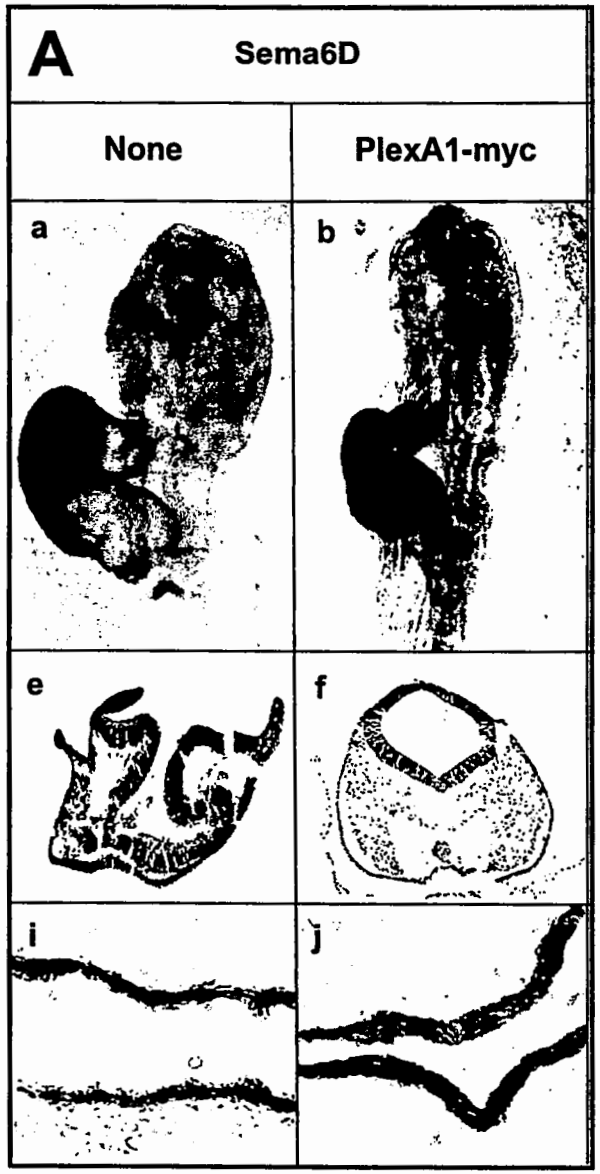


Truncated PlexinA1 blocks the effect of Sema6D

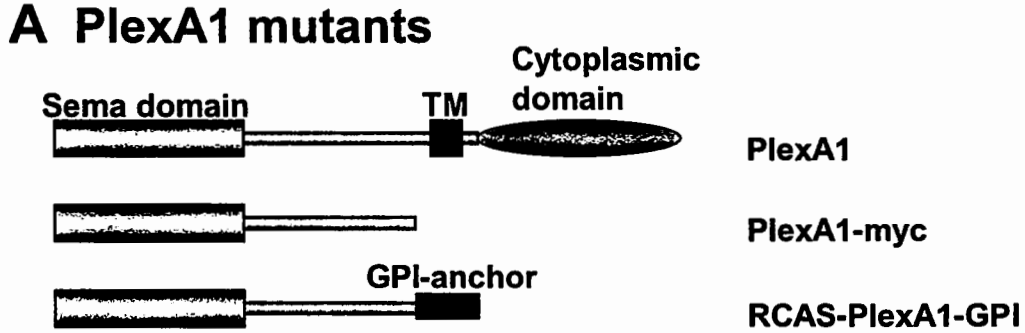
A PlexA1 mutants

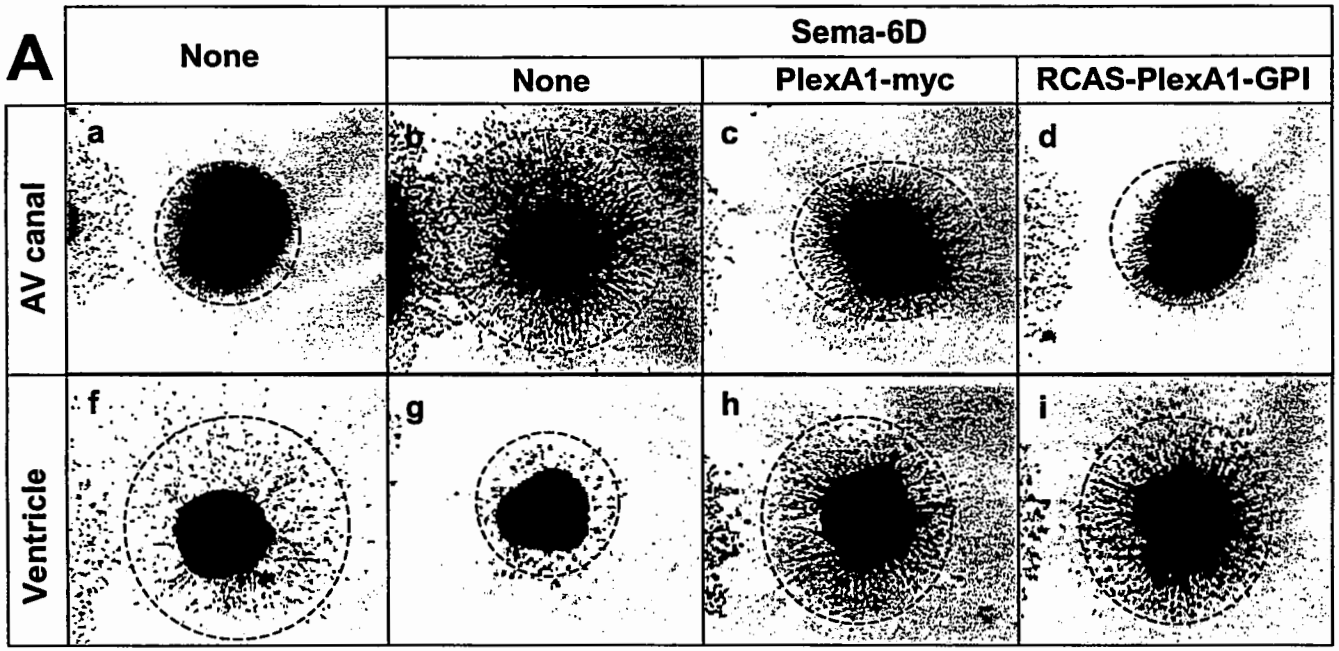


B Truncated PlexA1 suppresses Sema6D effects



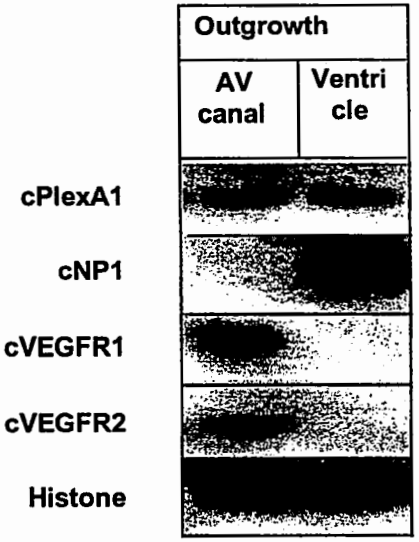
Truncated forms of Plexin A1 block effects of Sema6D on cardiac explants



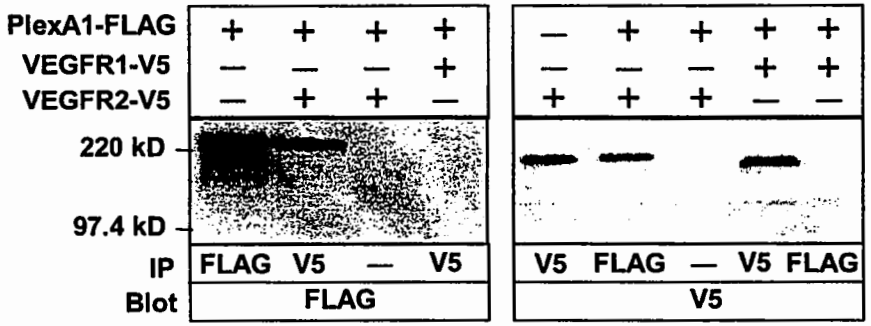


Plexin A1 can associate with VEGFR2

A Expression receptors in cardiac explants

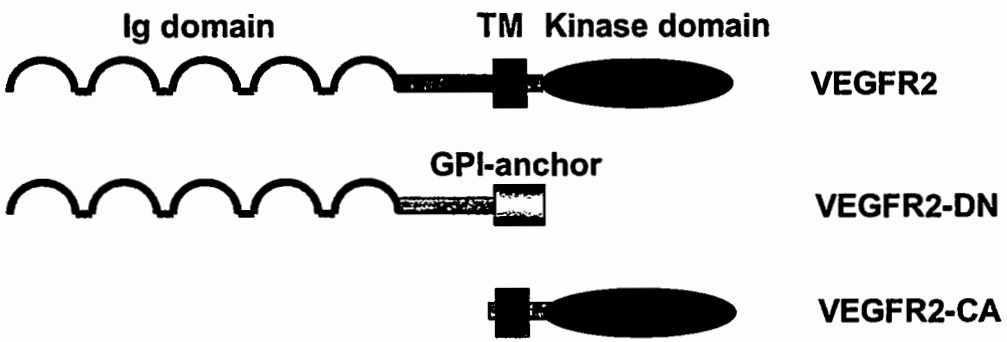


B PlexA1 associates with VEGFR2

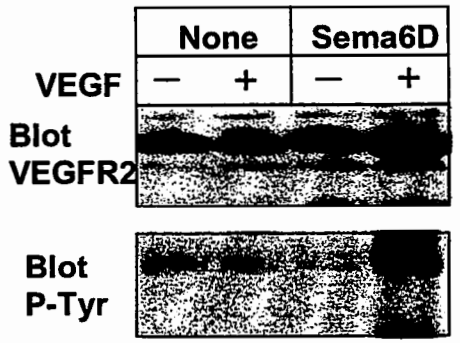


VEGFR2 is involved in Plexin A1 signals in AV canal

A VEGFR2 mutants



C Sema6D enhances VEGFR2 phosphorylation



B VEGFR2 mutants regulate Sema6D-induced Chemoattraction in AV canal