A requirement for neuropilin-1 in embryonic vessel formation

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SUMMARY

Neuropilin-1 is a membrane protein that is expressed in developing neurons and functions as a receptor or a component of the receptor complex for the class 3 semaphorins, which are inhibitory axon guidance signals. Targeted inactivation of the neuropilin-1 gene in mice induced disorganization of the pathway and projection of nerve fibers, suggesting that neuropilin-1 mediates semaphorin-elicted signals and regulates nerve fiber guidance in embryogenesis. Neuropilin-1 is also expressed in endothelial cells and shown to bind vascular endothelial growth factor (VEGF), a potent regulator for vasculogenesis and angiogenesis. However, the roles of neuropilin-1 in vascular formation have been unclear. This paper reported that the neuropilin-1 mutant mouse embryos exhibited various types of vascular defects, including impairment in neural vascularization, agenesis and transposition of great vessels, insufficient aorticopulmonary truncus (persistent truncus arteriosus), and disorganized and insufficient development of vascular networks in the yolk sac. The vascular defects induced by neuropilin-1 deficiency in mouse embryos suggest that neuropilin-1 plays roles in embryonic vessel formation, as well as nerve fiber guidance.

Key words: neuropilin-1, Mouse, Vascular anomaly, Neural vascularization, Branchial arch artery, Truncus arteriosus, Vascular endothelial growth factor

INTRODUCTION

Vascular endothelial growth factor (VEGF) is a major regulator of endothelial cell proliferation, vasculogenesis, angiogenesis and vascular permeability (Ferrara and Henzel, 1989; Keck et al., 1989; Leung et al., 1989; Carmeliet et al., 1996; Ferrara et al., 1996; for reviews, see Risau, 1997). The activities of VEGF are mediated by two endothelial cell-associated high-affinity receptor tyrosine kinases, VEGFR-1 (Flt-1; Shibuya et al., 1990; de Vries et al., 1992) and VEGFR-2 (KDR/Flk-1; Terman et al., 1991; Matthews et al., 1991; Millauer et al., 1993; Quinn et al., 1993). Gene-disruption studies have indicated that these two VEGF receptors play crucial roles in embryonic vessel formation (Fong et al., 1995; Shalaby et al., 1995, 1997).

Recently, neuropilin-1 was isolated from non-endothelial tumor cells as a protein that can bind VEGF165, a splicing isoform of the VEGF gene (Soker et al. 1998). Neuropilin-1 is a type 1 membrane protein, which is expressed in particular classes of developing neurons (Takagi et al., 1987, 1991, 1995; Kawakami et al., 1996) and functions as a receptor for the class 3 semaphorins mediating semaphorin-elicted inhibitory axon guidance signals to neurons (He and Tessier-Lavigne, 1997; Kolodkin et al., 1997; for a review, see Fujisawa and Kitsukawa, 1998). Neuropilin-1 is also expressed in endothelial cells of capillaries and blood vessels and endocardial cells of the heart (Kitsukawa et al., 1995). Coexpression of neuropilin-1 with VEGFR-2 in transfected cells enhanced VEGF165 binding to VEGFR-2 and VEGF-elicted mitogenic and chemotactic activities (Soker et al. 1998). Furthermore, we previously showed that overexpression of neuropilin-1 in mouse embryos resulted in an excess production of blood vessels and malformed hearts (Kitsukawa et al., 1995). These findings suggest that neuropilin-1 is the third endothelial cell-associated VEGF receptor to mediate or modulate the effects of VEGF and plays roles in vasculogenesis and angiogenesis. However, the function of neuropilin-1 in embryonic vessel formation has not been determined.

We previously produced neuropilin-1-null mutant mice by homologous recombination of the neuropilin-1 gene and indicated that neuropilin-1 deficiency in embryos induced...
disorganization of nerve pathways (Kitsukawa et al., 1997). The result suggests that neuropilin-1 plays essential roles in directional guidance of nerve fibers in embryonic development. The neuropilin-1 mutant was embryonic lethal and expected to suffer cardiovascular anomalies (Kitsukawa et al., 1997).

We here closely examined the vascular system of the neuropilin-1 mutant mouse embryos and report that neuropilin-1 deficiency induces various types of vascular defects. Vascularization in both the central nervous system (CNS) and peripheral nervous system (PNS) are severely impeded in the neuropilin-1 mutant embryos. The neuropilin-1 mutant embryos also exhibit agenesis of branchial arch-related great vessels and dorsal aorta, transposition of the aortic arch, insufficient septation of the truncus arteriosus and disorganized extraembryonic vasculature. The vascular regression in the embryos with neuropilin-1 deficiency is in contrast to the overproduction of vessels in the embryos with excess neuropilin-1 (Kitsukawa et al., 1995), suggesting that neuropilin-1 plays roles in embryonic vessel formation.

In addition, we performed chromosomal assignment for the neuropilin-1 gene, because the cardiovascular defect induced by neuropilin-1 deficiency in the mouse embryo resembles the human congenital cardiovascular defect that is often associated with microdeletions of chromosome 22q11 (for reviews, see Johnson et al., 1995; Lindsay and Baldini, 1998). The present study used mouse embryos produced by intercrossing heterozygous offspring created by back crossing germline chimeric mice with wild-type ICR three times. Noon on the day on which a copulation plug was found was designated as 0.5 day postcopulation (0.5 dpc).

**Detection of capillaries and blood vessels**

Capillaries and blood vessels in tissue sections were detected by the binding of isocitrate B4 (Ashwell, 1991). Pregnant mice were deeply anesthetized with nembutal (Dinabot), and then embryos were dissected out in Hanks’ solution. Embryos were fixed with 4% paraformaldehyde in 10 mM PBS (pH 7.0) overnight at 4°C, equilibrated with 0.5 M sucrose solution in PBS for 1 day and embedded in OCT compound (Tissue Tek, Miles). Sections (16 µm thick) were cut on a cryostat and collected on poly-L-lysine-coated glass slides, and incubated overnight at 4°C with biotine-labeled isolectin B4 (Ashwell, 1991). The chromosome slides were hardened for 2 hours at 65°C, denatured at 70°C in 70% formamide in 2x SSC, and dehydrated in a 70-85-100% ethanol series. The mouse neuropilin-1 cDNA fragment (1,122 base pairs corresponding to amino acids 483-856 of the neuropilin-1 protein; Kawakami et al., 1997) was inserted into pBluescript labeled with nick translation with biotin 16-dUTP (Boehringer Mannheim) following the manufacturer’s protocol. The labeled DNA fragment was ethanol-precipitated with salmon sperm DNA and E. coli tRNA and denatured in 100% formamide for 10 minutes at 75°C. The denatured probe was mixed with an equal volume of hybridization solution to make a final concentration of 50% formamide, 2x SSC, 10% dextran sulfate, and 2 mg/ml BSA (Sigma). 20 ml of mixture containing 250 ng of labeled DNA was put on the denatured chromosome slides, covered with parafilm and incubated overnight at 37°C. The slides were washed in 50% formamide in 2x SSC for 20 minutes at 37°C, and then in 2x SSC and 1x SSC for 20 minutes each at room temperature. After rinsing in 4x SSC, the slides were incubated with anti-biotin antibody (Vector Laboratories; 1:500 dilution in 1% BSA/4x SSC) for 1 hour at 37°C under coverslips. The slides were washed with 4x SSC, 0.1% Nonidet P-40 (NP-40) in 0x SSC and 1x SSC for 5 minutes each, and then stained with fluorescein-conjugated anti-goat IgG (Nordic Immunology; 1:500 dilution) for 1 hour at 37°C. After washing with 4x SSC, 0.1 % NP-40 in 4x SSC and 4x SSC for 5 minutes each, the slides were incubated with anti-biotin antibody (Vector Laboratories; 1:500 dilution in 1% BSA/4x SSC) for 1 hour at 37°C under coverslips. The slides were washed with 4x SSC, 0.1% Nonidet P-40 (NP-40) in 0x SSC and 4x SSC for 5 minutes each, and then stained with fluorescein-conjugated anti-goat IgG (Nordic Immunology; 1:500 dilution) for 1 hour at 37°C. After washing with 4x SSC, 0.1 % NP-40 in 4x SSC and 4x SSC for 10 minutes each on the shaker, the slides were rinsed with 2x SSC and stained with propidium iodide (0.75 µg/ml). Excitation at wave length 450-490 nm (Nikon filter B-2A) and near 365 nm (UV-2A) were used for observation.
RESULTS

Impairment of neural vascualization
A typical vascular phenotype of the neuropilin-1 mutant was the impairment of neural vascualization. In both the wild-type (NP-1+/+) and heterozygous neuropilin-1 mutant (NP-1+/−) embryos, vascular sprout from the perineural vascular plexus vessels (leptomeningeal arteries and veins) into the CNS took place at around 9 dpc in the ventral parts of the mesencephalon and metencephalon (data not shown), and then expanded to most parts of the brain at 10.5 dpc, including the ventral telencephalon (Fig. 1A), diencephalon (Fig. 1C), mesencephalon (Fig. 1E) and metencephalon. In contrast, capillary invasion was absent in the CNS from homozygous neuropilin-1 mutant (NP-1−/−) embryos at 10.5 dpc (Fig. 1B,D), except in the ventral part of the mesencephalon (Fig. 1F) and metencephalon, even though the CNS anlage was invested by several blood vessels.

Impaired neural vascualization was also apparent in the NP-1−/− embryos at 12.5 dpc. The neocortex (Fig. 2B), the dorsal part of the midbrain (Fig. 2F) and spinal cord (Fig. 2J), sensory ganglia such as the spinal ganglia (Fig. 2J) and trigeminal ganglia (Fig. 2L) showed little vasculalization. Though a considerable number of capillaries were detected in the diencephalon (Fig. 2D), ventral midbrain (Fig. 2F), hindbrain (Fig. 2H) and ventral spinal cord (Fig. 2J), they had few branches, were large in caliber, and were often broken into small spherical segments (Fig. 2D,F,H,J). Staining of tissue sections with Hematoxylin-Eosin revealed degenerating capillaries containing blood cells (Fig. 3A). The reduction of neural vasculalization induced neuronal cell death and subsequent tissue destruction in embryos at 12.5 dpc (Fig. 3B). By 13.5 dpc, most NP-1−/− embryos had died, but a few embryos with severe edema were still alive.

Impaired neural vascualization was observed in all NP-1−/− embryos examined (5 and 8 embryos at 10.5 dpc and 12.5 dpc, respectively) but not in the NP-1+/+ embryos (3 and 5 embryos at 10.5 dpc and 12.5 dpc, respectively). The regional difference in the impairment of neural vasculalization described above did not show any correlation to the expression patterns of neuropilin-1 within the nervous system (see Kawakami et al., 1996).

Defects in great vessels
In embryonic development, 5 pairs of branchial arch arteries (the 1st-4th and 6th arch arteries) successively differentiate in relation to their respective branchial arches, as the caudal vessels become apparent, the most rostral vessels largely disappear and the definitive pattern of great
vessels is established (Kaufman, 1992). The development of the branchial arch arteries and great vessels was disorganized in the neuropilin-1-deficient mouse embryos.

All of the NP-1+/− embryos examined at 12.5 dpc (21 embryos from 10 dams) but not the NP-1+/+ or NP-1+/− littersmates (108 embryos in sum) lacked the left 6th arch artery that normally forms the ducts arteriosus Botalli and proximal part of the pulmonary arteries (see Fig. 4B-F). Besides this defect, the NP-1−/− embryos exhibited various anomalies in the great vessels, which were subdivided into three major types. The first and most frequent variant (in 15 out of 21 embryos) was that the left 4th branchial arch artery, which normally formed the aortic arch on the left side of body (see Fig. 4A,G), was absent and the aortic arch was formed on the right side instead (the right-sided aortic arch; Fig. 4B,C,H). The right-sided aortic arches were often located at a position higher than that for the original right 4th arch artery (the high position of aortic arch). The left subclavian artery arose from the right-sided descending aorta (Fig. 4D). The second variant (in 3 out of 21 embryos) was that the aortic arch was formed on the left side of the body (Fig. 4D,E) as in normal embryos. In one embryo, the position of the aortic arch was higher than that for the 4th branchial arch artery, and the right 4th arch artery was absent (Fig. 4D). In this embryo, the right subclavian artery arose from the left-sided descending aorta. The third variant (in 3 out of 21 embryos) was that the aortic arch was formed on both sides of body (the double aortic arch; Fig. 4F,I).

In 1 embryo, the position of both the left and right aortic arches was in high (Fig. 4F). The left and right subclavian arteries arose from the left and right descending aortas, respectively (Fig. 4I). In all NP-1−/− embryos at 12.5 dpc, both the left and right pulmonary arteries toward the primitive lung buds emerged directly from the proximal part of the aortic arch (Fig. 4I), even though the embryos lacked the left 6th arch-related artery. The vertebral arteries and intersegmental arteries were normally formed in the neuropilin-1 mutant embryos (data not shown).

The formation of the branchial arch arteries and dorsal aorta was abnormal from the beginning of development in the neuropilin-1 mutants, even though branchial arches were normally formed. In the NP-1+/+ and NP-1+/− embryos at 10.5 dpc (51 embryos in sum from 6 dams) the 3rd, 4th and 6th arch arteries were formed in a bilaterally symmetrical pattern and connected with the left or right dorsal aortas (Fig. 5A,E). In contrast, all NP-1−/− embryos examined (22 embryos from 6 dams) lacked the 6th arch artery of both sides (Fig. 5B-D,F-M), and 15 out of them further lacked the 4th arch artery of both sides (Fig. 5B-D,F-H,I,J). Some embryos also lacked the 3rd arch artery on either side (Fig. 5B-D,H,K,M). At 10.5 dpc, the 1st and 2nd branchial arch arteries had mostly been involuted in the NP-1+/+ and NP-1+/− embryos (Fig. 5A,E), but often remained in the NP-1−/− embryos (Fig. 5C,D,G-K,M). At 9.5 dpc, all NP-1+/+ and NP-1−/− embryos (19 embryos from 2 dams) possessed the 1st, 2nd and 3rd arch arteries in a bilaterally symmetrical pattern (Fig. 5N). In contrast, all NP-1−/− embryos (5 embryos from 2 dams) exhibited a severe regression of the arch artery system (Fig. 5O-Q). Segments of dorsal aorta were also regressed in most NP-1−/− embryos at 10.5 dpc (Fig. 5B,C,F,G,I,K-M) and 9.5 dpc (Fig. 5O-Q).

The disorganized development of the branchial arch artery system and the partial regression of dorsal aorta may result in the abnormal and highly variable patterns of great vessels in embryos at 12.5 dpc. The lack of the 4th arch artery of both sides in most neuropilin-1 mutant embryos suggests that the remaining 3rd or sometimes 2nd arch arteries take part in the formation of the aortic arch at high positions.

Defect in heart outflow tracts

The development of heart outflow tracts was disturbed in the neuropilin-1 mutant embryos. In the NP-1+/+ and NP-1+/− embryos at 12.5 dpc, the aorticopulmonary truncus had been separated into the aortic and pulmonary channels (Fig. 6A,B,D,E). In contrast, separation of the truncus arteriosus was

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**Fig. 4.** Cardiovascular system of the wild-type (+/+) and homozygous neuropilin-1 mutant (−/−) mouse embryos at 12.5 dpc. Great vessels and hearts were filled with Indian ink. (A-F) Ventral view. An arrow in A indicates the ducts arteriosus. (G-I) Ventral view of great vessels (heart has been removed). Arrowsheads indicate the left subclavian arteries. (J) Lateral view of the heart (left and right atriums have been removed). la, left atrium; ra, right atrium; lv, left ventricle; rv, right ventricle; cca, common carotid artery; pa, pulmonary artery. Scale bar, 500 μm; a bar in A for A-I.
incomplete (persistent truncus arteriosus) in most NP-1−/− embryos at 12.5 dpc (Fig. 6C). As the left 6th arch artery did not develop in the mutant embryos, the distal end of the pulmonary channel merged with the aortic arch (Fig. 6C). In a few NP-1−/− embryos, the truncus arteriosus was not separated at all and persisted as a single outflow vessel of the heart (Fig. 6F,G). The formation of the interventricular septum in the NP-1−/− embryos was apparently normal (compare Fig. 6A and C).

Vascular defects in the yolk sac
Organization of vascular networks in the yolk sac was also abnormal in the neuropilin-1 mutant embryos, even though the yolk sacs of the mutant embryos were well vascularized as that of the wild-type embryos. In all NP-1−/− embryos examined at 12.5 dpc, large vessels were meandering and often divided into small vessels that anastomosed (Fig. 7B). Moreover, whole-mount immunostaining of yolk sacs with an anti-PECAM antibody showed that capillary networks were sparse in the NP-1−/− embryos in comparison to the NP-1+/+ or NP-1+/− embryos (compare Fig. 7C and D).

Assignment of a chromosome for the neuropilin-1 gene
In the human, microdeletions of chromosome 22q11 are often associated with cardiovascular abnormalities such as regression and/or transposition of branchial arch-related great vessels and persistent truncus arteriosus (for reviews, see Johnson et al., 1995; Lindsay and Baldini, 1998), which are induced by neuropilin-1 deficiency in mice. Moreover, a neuropilin-1 homologue has been identified in the human (He and Tessier-Lavigne, 1997; Kolodkin et al., 1997). Therefore, we performed chromosomal assignment of the neuropilin-1 gene by direct R-banding FISH using the mouse neuropilin-1 cDNA fragment as a probe.

The neuropilin-1 gene was localized at E region of mouse chromosome 8 (Fig. 8A,B; for the pattern of mouse chromosome banding, see Matsuda et al., 1992; Somssich and Hameister, 1996) and q12.1 of rat chromosome 19 (Fig. 8C,D; for the pattern of rat chromosome banding, see Satoh et al., 1989), where conserved linkage homology to mouse chromosome 8 but not to the human 22q11 has been identified (Yamada et al., 1994; Lyon et al., 1997; Serikawa et al., 1998).

DISCUSSION
The present study showed that neuropilin-1 deficiency in mouse embryos induced severe anomalies in the vascular system, and provides evidences of the involvement of neuropilin-1 in embryonic vessel formation.

Neuropilin-1 functions as a receptor or a component of receptor complex for the class 3 semaphorins (He and Tessier-Lavigne, 1997; Kolodkin et al., 1997), and a deficiency of neuropilin-1 in mouse embryos induced disorganization in the pathways and projections of PNS efferent fibers (Kitsukawa et al., 1997). A similar neuronal phenotype to that in the neuropilin-1 mutant is observed in a mouse strain in which a ligand for neuropilin-1, Semahorin3A (Sema3A; a new nomenclature for mouse Semaphorin D; Semaphorin Nomenclature Committee, 1999), was inactivated (Taniguchi et al., 1997). However, it seems unlikely that the interception of neuropilin-1-mediated Sema3A activities results in the present vascular defects, because the Sema3A mutant strain

![Fig. 5. Cardiovascular system of the wild-type (+/+ and homozygous neuropilin-1 mutant (−/−) mouse embryos at 10.5 dpc and 9.5 dpc. (A-D) Great vessels and hearts from embryos at 10.5 dpc were filled with Indian ink. Each figure indicates the left-side view (le) and right-side view (ri) of the heart (h), branchial arch arteries and dorsal aorta (da). The 1st to 6th branchial arch arteries are indicated by the numbers 1 to 6. (E-M) Schematic representation of the branchial arch artery system of the wild-type (E) and NP-1−/− (F-M) embryos at 10.5 dpc. The dotted lines indicate undifferentiated or regressed arteries. (N-Q) Schematic representation of the branchial arch artery system of the wild-type (N) and NP-1−/− (O-Q) embryos at 9.5 dpc. Scale bar, 1 mm in A for A-D.](image-url)
shows no apparent vascular anomalies (M. Taniguchi, personal communication), and furthermore, another Sema3A mutant mouse strain (Behar et al., 1996) shows hypertrophy of the right ventricle of the heart and dilation of the right atrium, but no anomalies in great vessels, heart outflow tracts and neural vasculization. However, neuropilin-1 can also bind VEGF165, a potent mitogen for endothelial cells (Soker et al., 1998). A more recent study showed that neuropilin-1 functions as a receptor for another VEGF-family growth factor, placenta growth factor-2 (Migdal et al., 1998). Therefore, it seems more likely that the vascular defect in the neuropilin-1 mutant mouse embryos is primarily attributable to the interception of VEGF-family growth factor activities.

It has been shown that neuropilin-1 enhances the binding of VEGF to its receptor VEGFR-2 (KDR/Flk-1) and increases mitogenic activity and chemotaxis, even though neuropilin-1 itself shows no such bioactivities (Soker et al., 1998). Mouse embryos deficient in VEGFR-2 (Flk-1) show a severe defect in the formation of blood islands and disorganized assembly of endothelial cells into functional vessels and die at 8.5 dpc (Fong et al., 1995; Shalaby et al., 1995). In contrast, the vascular phenotype in the neuropilin-1-deficient mouse embryos is more moderate than that for the VEGFR-2-deficient mice. The neuropilin-1 mutant embryos exhibit a partial impairment in neural vasculization, hypoplasia of segments of the arch arteries and dorsal aorta, and a partial disorganization in the extraembryonic vasculature, but no major defects in the differentiation of endothelial cells or in the formation of primary vascular networks. The difference in vascular phenotype between the VEGFR-2 mutant and the neuropilin-1 mutant suggests that VEGFR-2-mediated VEGF...
activities are prerequisite for vasculogenesis and embryonic vessel formation, and the activation of VEGF activities by neuropilin-1 is required for the maturation and remodeling of the primary vascular network into the definitive embryonic and extraembryonic vasculatures and for early development of the cardiovascular system.

Targeted inactivation of one allele of the VEGF gene in mice disturbs embryonic vascular formation (Carmeliet et al., 1996; Ferrara et al., 1996), suggesting that development of embryonic vessels requires a tight dose-dependent regulation by VEGF (Ferrara et al., 1996). The regression of neural vascularization in the neuropilin-1-deficient mouse embryos contrasts to the excess production of vessels in the nervous tissues of mouse embryos with neuropilin-1 overexpression (Kitsukawa et al., 1995). We therefore infer that, in the vascular system, neuropilin-1 functions as a regulator to adjust VEGF activities at minimally required levels. The gain- or loss-of-function of neuropilin-1 in embryos may upregulate or downregulate VEGF activities, resulting in the excess production or regression of embryonic vessels.

In the CNS, the ventricular layer is the origin of VEGF (Breier et al., 1992). In normal embryos, vascular sprout from the perineural vascular plexus vessels into the CNS is initiated at 9 dpc in the ventral parts of the mesencephalon and metencephalon, and then expands to other parts of the brain. These findings suggest that the level of VEGF-expression is different from region to region within the ventricular layer and determines the timing and extent of vascularization into neural tissues. In the neuropilin-1 mutant embryos, capillary invasion into the CNS also took place in the ventral parts of the mesencephalon and metencephalon (see Fig. 1F) but delayed more than 1 day in comparison to the normal embryos, and then expanded through the CNS. The capillaries, however, were not organized into networks within the wall of the CNS and degenerated. The reduction in the response of neuropilin-1-deficient endothelial cells to VEGF may result in the delay of vascular sprout into neural tissues and the impaired capillary network formation. The regression of capillary networks and abnormal morphology in large vessels within the yolk sac may also be induced by the reduction of VEGF signals in the neuropilin-1-deficient endothelial cells.

The regression of branchial arch artery system and subsequent abnormal morphogenesis in the cardiovascular system that occurred in the neuropilin-1-deficient embryos seem to be induced by the reduction of VEGF signals in endothelial cells. However, several studies have indicated that the cardiac neural crest located at the level of occipital somites 1-3 provides cells in the tunica media of the wall of the 3rd, 4th and 6th arch arteries and cells in the aorticopulmonary septum (Le Lievre and Le Douarin, 1975; Noden, 1991; Miyagawa-Tomita et al., 1991; Waldo and Kirby, 1993; Waldo et al., 1998) and plays crucial roles in the development of cardiovascular system. Embryos in which the cardiac neural crest was surgically or chemically extirpated (Nishibatake et al., 1987; Miyagawa and Kirby, 1989; Okishima et al., 1992) or mutant mouse strains in which defects occurred in the cardiac neural crest (Mendelsohn et al., 1994; Kurihara et al., 1995; Conway et al., 1997) show a similar cardiovascular anomaly to that for the neuropilin-1-deficient mouse embryos, including the regression of the 6th arch artery, right-sided aortic arch or persistent truncus arteriosus. Therefore, the cardiovascular anomaly in the neuropilin-1 mutant mouse embryos might be attributable in part to inappropriate migration and/or differentiation of cardiac neural crest cells. As described above, the Sema3A mutants show no apparent vascular defect. However, the cardiac neural crest cells from chick embryos express neuropilin-1, and their migration is regulated by Sema3A (collapsin-1; Eickfolt et al., 1999). Moreover, Sema3A is strongly expressed in the embryonic heart (Taniguchi et al., 1997). These lines of observation suggest that deprivation of neuropilin-1 from the cardiac neural crest cells disrupts the transduction of Sema3A activities into the cells and results in incorrect cell migration or differentiation. VEGF165 functions as an antagonist for Sema3A and inhibits the binding of Sema3A to neuropilin-1 and vice versa (Giger et al., 1998; Miao et al., 1999). Therefore, it is also possible that VEGF regulates the migration or differentiation of cardiac neural crest cells via direct interaction with neuropilin-1 or suppression of Sema3A-neuropilin-1 interaction. Though further analyses are required to clarify the roles of semaphorins and VEGF in the migration and differentiation of cardiac neural crest cells, neuropilin-1 seems to be a key molecule regulating cardiovascular embryogenesis.

The cardiovascular defect induced in the mice deficient in neuropilin-1 resembles that in humans with microdeletions of chromosome 22q11, even though the present chromosomal assignment for the neuropilin-1 gene and a more recent mapping of the human NEUROPILIN-1 gene (Rossignol et al., 1999) indicate that the gene is not localized at 22q11. The neuropilin-1 mutant mouse seems to be a good animal model to analyze the pathogenesis of congenital cardiovascular malformation.

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