Int. J. Dev. Biol. 52: 87-92 (2008)
doi: 10.1387/ijdb.072374rr

THE INTERNATIONAL JOURNAL OF DEVELOPMENTAL BIOLOGY
www.intjdevbiol.com

Stromal-derived factor-1 *(SDF-1)* expression during early chick development

RIZWAN REHIMI[1,2], NARGIS KHALIDA [1], FAISAL YUSUF[1], FANGPING DAI[1], GABRIELA MOROSAN-PUOPOLO[1,2] and BEATE BRAND-SABERI[1,*]

[1] *Institute of Anatomy and Cell Biology, Department of Molecular Embryology and* [2] *Faculty of Biology, University of Freiburg, Freiburg, Germany*

ABSTRACT Cell migration plays a fundamental role in a wide variety of biological processes including development, tissue repair and disease. These processes depend on directed cell migration along and through cell layers. Chemokines are small secretory proteins that exert their effects by activating a family of G-protein coupled receptors and have been shown to play numerous fundamental roles in the control of physiological and pathological processes during development and in adult tissues, respectively. Stromal-derived factor-1 (SDF-1/CXCL12), a ligand of the chemokine receptor, CXCR4, is involved in providing cells with directional cues as well as in controlling their proliferation and differentiation. Here we studied the expression pattern of SDF-1 in the developing chick embryo. We could detect a specific expression of SDF-1 in the ectoderm, the sclerotome, the intersomitic spaces and the developing limbs. The expression domains of SDF-1 reflect its role in somitic precursor migration and vessel formation in the limbs.

KEY WORDS: *SDF-1, CXCR4, cell migration, chick embryo, differentiation*

Introduction

The role of cell migration is of paramount importance in numerous biological processes occuring during development and disease. During development, many cells actively migrate from one place to another where they differentiate into mature tissues and organs. Cell migration is a complex process involving dynamic interactions between migrating cells and tissues through which they migrate. In order to migrate and invade a target tissue, cells change shape and adhesion properties. Cells are able to read the guidance cues provided by target tissues that tell them where to go and when to stop. There are some complex groups of regulators that direct cell movement by modulating adhesion, attraction and repulsion. The foremost regulators of chemoattraction are members of the chemokine family (Luster 1998, Kim and Broxmeyer 1999). Chemokines are small secretory proteins that exert their effects by activating a family of seven-pass transmembrane G-protein coupled receptors that typically recognize a wide variety of ligands and have been shown to play numerous fundamental roles in the control of physiological and pathological processes. Due to the large number of chemokines and rapid progress within the field, many chemokines have been reported by different research groups and have been given multiple names. Chemokines are able to bind to multiple chemokine receptors, but until recently, stromal derived factor (SDF-1) was known to bind exclusively to chemokine receptor CXCR4 and that in turn also binds solely to SDF-1. This however has changed after finding from the work of groups that could show that SDF-1 can also bind and signal through a previously known orphan receptor RDC1/CXCR7 (Balabanian *et al.*, 2005; Dambly-Chaudiere *et al.*, 2007). These findings open up more possibilities for SDF-1 signalling which as yet was only thought to occur via CXCR4.

The chemokine and its receptor pair, CXCR4/SDF-1 are well investigated in developmental process such as in vascularization, embryogenesis, T-cell activation and migration at sites of inflammation and T-Cell homing, hematopoiesis and HIV pathogenesis. (Murdoch 2000; Balkwill 2004). During brain development, *SDF-1* plays a very critical role in several aspects such as cell migration and axon pathfinding, but recently its role to stimulate axonal branching and to regulate axonal patterning has been described (Pujol *et al.*, 2005).

Stromal cell-derived factor (*SDF-1*) is a member of the CXC

Abbreviations used in this paper: SDF, stromal cell-derived factor.

***Address correspondence to:** Prof. Dr. Beate Brand-Saberi. Institute of Anatomy and Cell Biology, Department of Molecular Embryology, Albert-Ludwig University, Albertstrasse 17, 79104 Freiburg, Germany. Fax: +49-761-2035-387. e-mail: beate.brand-saberi@anat.uni-freiburg.de

Accepted: 13th June 2007. *Published online:* 8th November 2007. *Edited by:* Patrick Tam.

0214-6282/2008/$35.00
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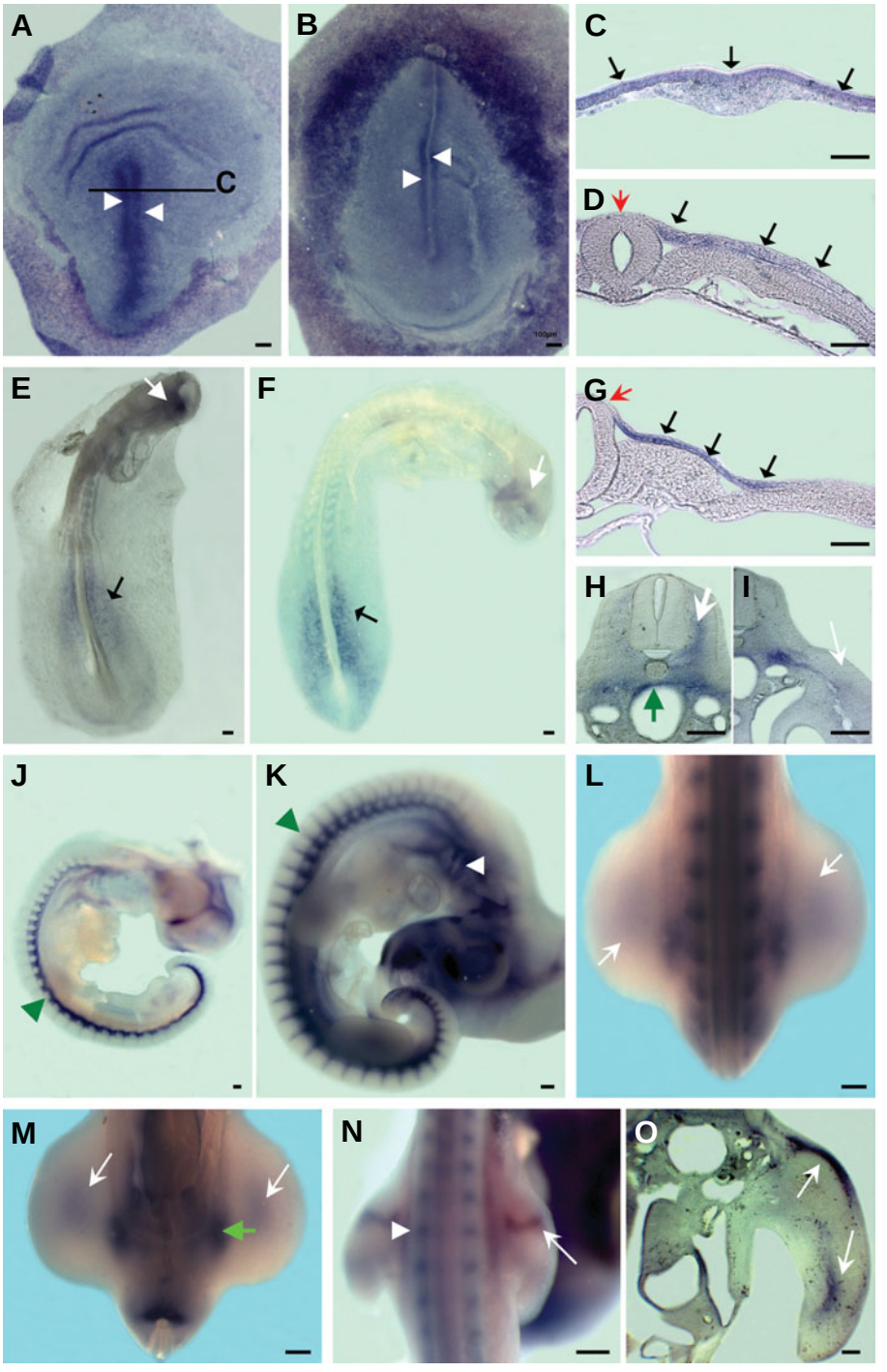

subfamily of chemokines. *SDF-1* is expressed in many organs including bone, lung, liver, brain, thymus and lymph nodes (Shirozu *et al.*, 1995, Nagasawa *et al.*, 1998), but it is mainly produced by stromal cells such as osteoblasts, fibroblasts and endothelial cells in the bone marrow (Yun *et al.*, 2003). Initially, *SDF-1* was cloned from murine bone marrow and characterized as pre-B cells growth stimulating factor (Nagasawa *et al.*, 1994, Shirozu *et al.*, 1995). Physiological functions of chemokines have been described as *SDF-1* plays a very important role in the migration of germ cells. During development these cells undergo a very specialized process of migration from their site of origin to the future gonad. Chemokine *CXCR4/SDF-1* mutant germ cells in zebrafish are unable to migrate directly towards their target tissue from their site of origin, this result indicating that ligand SDF-1 gives the guiding cues to migratory germ cells at all stages of migration towards their target (Knaut *et al.*, 2003). In *SDF-1*(-/-) mice, PGCs migrate normally through tissues of embryos, but the numbers of PGCs in the gonads are remarkably reduced. These findings revealed an aberrant colonization of the gonads by germ cells lacking a functional chemokine ligand and receptor (Ara *et al.*, 2003). Further evidence for the role of this pair during germ cell migration in mice and avian comes from the work of Stebler and collegues, who could show the relevance of the *SDF-1* expression to the primodial germ cells migratory pathway (Stebler *et al.*, 2004). The *SDF-1*/*CXCR4* axis is also required for normal myelopoiesis and lymphopoiesis (Murdoch 2000). Recently, we have reported the first use of a peptidic inhibitor for *CXCR4* in the developing chick embryo and observed that cells migrating from the dermomyotome into the limb bud acquire an angiogenic fate, whereas the myogenic fate is repressed, implying that *SDF-1* signalling is directly or indirectly involved in the myogenic determination of the *CXCR4* expressing cells in the limb (Yusuf *et al.*, 2006).

Many severe defects have been observed during development in knockout studies of

Fig. 1. *In situ* hybridization analysis of *SDF-1* in chick embryo from stage HH3 to HH25. (A-O) *Expression of* SDF-1 *in developing chick embryos. At stages HH3-5, strong expression is noted at the level of the primitive streak (white arrowheads in A and B).* **(C)** *Section of (A) at the level of the primitive streak, showing expression in the epiblast (black arrows).* **(E,F)** *Stages HH12-14. Prominent ectodermal expression can be observed in the caudal region of the embryo (black arrows), whereas more cranially,* SDF-1 *is not expressed. Also note the expression domains in the head region (white arrows).* **(D,G)** *Sections of stage HH12 and HH14 embryos respectively, showing* SDF-1 *signal overlying the paraxial mesoderm, intermediate mesoderm and medial parts of lateral plate (black arrows). However, the signal is missing in the ectoderm overlying the neural tube (red arrows).* **(J,K)** *Stages HH18^{+}-20^{+}; expression in the intersomitic spaces (green arrowhead) as well as in the branchial arches can be seen (white arrowhead in K).* **(H)** *Cross section of interlimb region of HH20^{+} showing a prominent expression domain around the dorsal aortic wall (green arrow) and sclerotome (white arrow). Sclerotomal staining also observed at HH25 (white arrowhead in N).* **(I,L,M,N)** *Stages 20^{+}-25. Transcripts of* SDF-1 *are observable in the limbs (white arrow).* **(M)** *Expression domain in the cloacal region (green arrow).* **(O)** *Transverse section of limb, displaying restricted expression domains in the limb and mesenchyme (white arrows). The scale bar represents 100 μm in each photo.*

CXCR4 or its ligand *SDF-1*, such as cardiac ventricular defects, aberrations of intestinal vasculature and several affected hemopoietic compartments (Zou *et al.*, 1998). Animals, deficient for either *CXCL12* or *CXCR4,* exhibit severe aberration of cerebellar and hippocampal morphology, defects currently believed to result from the disconcerted migration of neuronal precursors (Zou *et al.*, 1998, Zhu *et al.*, 2002; Bagri *et al.*, 2002; Lu *et al.*, 2002). *CXCL12* is also known to modulate axonal pathfinding (Xiang *et al.*, 2002; Chalasani *et al.*, 2003).

Interactions between *SDF-1* and its receptor *CXCR4* are involved in cell progression and metastasis of several cancers, including pancreatic cancer (Koshiba *et al.*, 2000), kidney cancer (Schrader *et al.*, 2002), prostate cancer (Taichman *et al.*, 2002) lung and ovarian cancer (Scotton *et al.*, 2001, 2002; Kijima *et al.*, 2002). Muller reported that *CXCL12*|*CXCR4* have been associated with metastasis of breast cancer (Muller and Neville 2001). In mice, the expression patterns of stromal cell-derived factor-1 and its receptor are also particularly noticeable in the developing cardiac, vascular and craniofacial system during organogenesis (McGrath *et al.*, 1999). *CXCR4* receptor is widely expressed in various tissues of the developing chick embryo such as in the ventral forgut portal, developing somites, tailbud, Wolffian duct, the lateral plate mesoderm and developing blood vessels (Yusuf *et al.*, 2005). In our study we documented the normal expression pattern of *CXCL12* (*SDF-1*) in chick embryos and we observed that *SDF-1* is widely expressed in most of the embryonic tissues during development such as in the ectoderm, the sclerotome, the intersomitic spaces and the mesenchyme of the developing limbs. Although the expression profile in *SDF-1* has been reported in context of primordial germ cell migration (Stebler *et al.*, 2004), we present the expression analysis of this important developmental gene in a broader context especially, highlighting its role in limb myogenesis and vasculogenesis and somitogenesis.

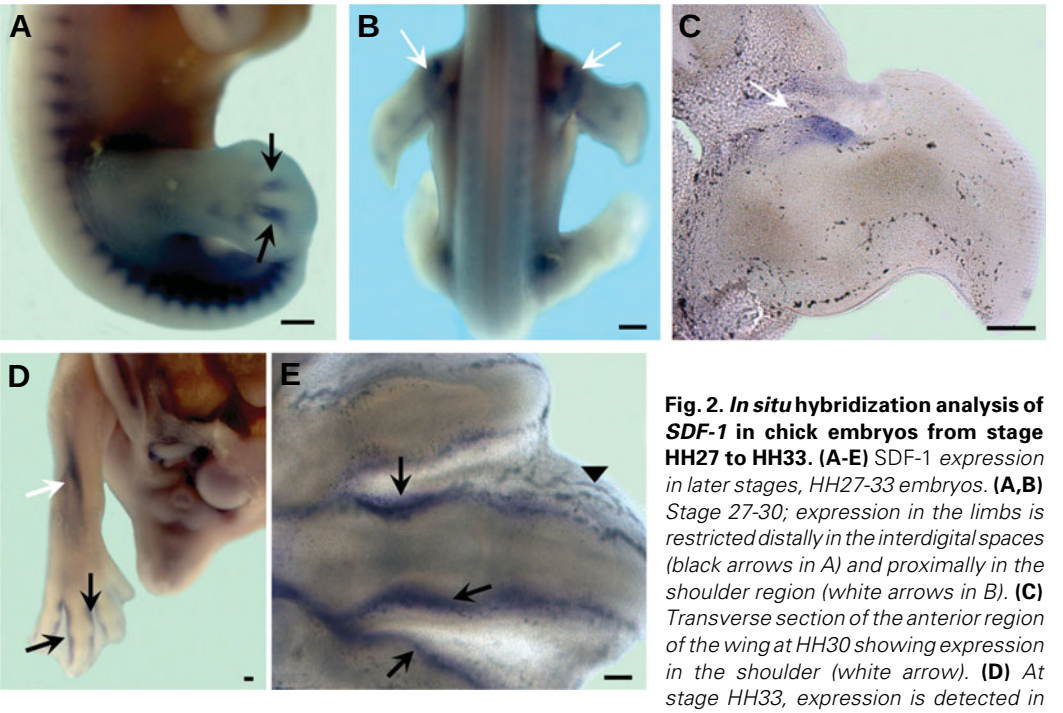

Fig. 2. *In situ* hybridization analysis of *SDF-1* in chick embryos from stage HH27 to HH33. (A-E) SDF-1 *expression in later stages, HH27-33 embryos.* **(A,B)** *Stage 27-30; expression in the limbs is restricted distally in the interdigital spaces (black arrows in A) and proximally in the shoulder region (white arrows in B).* **(C)** *Transverse section of the anterior region of the wing at HH30 showing expression in the shoulder (white arrow).* **(D)** *At stage HH33, expression is detected in the leg (white arrow in D), perichondral tissue (black arrows in D and E-section of distal limb) and in the blood vessels of the interdigital spaces (black arrowhead). The scale bar represents 100 μm in each photo.*

Results

Transcripts of *SDF-1* can be detected as early as stage HH3-5 in the chick embryos (Fig. 1A, B). The gastrulating embryo (Fig. 1A, B) stained for *SDF-1* intensely in the epiblast layer (Fig. 1C). At the level of the primitive streak, where the epiblast cells invaginate, staining is intense in the epiblast and mild in the underlying newly formed mesoderm (Fig. 1C), whereas more posteriorly, where gastrulation has not advanced yet, the epiblast stains faintly for *SDF-1* (not shown).

At stage HH12-15, the *SDF-1* transcripts are to be found predominantly in the ectoderm. Whole mounts for stages HH12 and HH14 show a prominent ectodermal staining in the caudal parts of the embryos and a faint signal in the head region is also detectable (Fig. 1 E, F). Sections revealed the ectodermal presence of *SDF-1* signal overlying the paraxial mesoderm, intermediate mesoderm and medial parts of the lateral plate mesoderm at stage HH12-14, however, the signal is missing in the ectoderm above the neural tube (Fig. 1D, G). Interestingly, the ectodermal presence of *SDF-1* seems to follow a gradient pattern along the antero-posterior axis. The unsegmented paraxial mesoderm is seen to be always covered by an *SDF-1* expressing ectoderm, but more cranially, the ectoderm overlying mature epithelial somites is devoid of *SDF-1* transcripts. No *SDF-1* expression can be seen in the underlying neural tissue or mesodermal tissue at these stages (Fig. 1 D, G).

A radical shift of *SDF-1* expression pattern is observed from stage HH18 onwards, where the transcripts are no longer detectable in the ectoderm, but are now predominantly seen in the mesodermal derivatives. At stage HH18^{+} whole mounts, one can detect a symmetrically arranged expression pattern in the intersomitic spaces and in the sclerotome, which is also visible on sections (Fig. 1J, H). High amounts of *SDF-1* transcripts are also visible around the dorsal aorta wall (Fig. 1H). At HH20^{+}, the intersomitic and sclerotomal expression persists (Fig. 1K), in addition, an expression domain in the branchial arches (Fig. 1K), in the limb bud (Fig. 1I, L, M) and the cloacal region is visible (Fig. 1M). The intersomitic expression domain persists at HH25 (Fig. 1N). The *SDF-1* transcripts at HH25 display restricted expression domains in the limb mesenchyme, one is situated proximally, dorsally and subectodermally (Fig.1 N, O) while the other located more or less in the center

of the distal 2/3 region of the limb (Fig. 1O).

As the limbs develop, the *SDF-1* expression domain gets restricted to the interdigital zones at HH27 (Fig.2 A) distally and proximally in the shoulder region at HH30 (Fig. 2B). Transverse section at the anterior end of the wing bud reveals the expression zone in the shoulder region (Fig. 2C). The interdigital expression domains at HH33 get limited to the perichondral tissue (Fig. 2 D, E). Control embryos hybridized using the sense probe showed no staining (data not shown).

Discussion

SDF-1 is highly conserved in the entire animal kingdom (Bleul *et al*., 1996). *SDF-1* was till recently known to bind exclusively to chemokine receptor, *CXCR4*, as its sole ligand (McGrath *et al*., 1999, Murdoch 2000), however, finding have now shown that it also can bind and signal through *RDC1/CXCR7*. Stebler and group have previously reported the functionally relevant expression of *SDF-1* and its receptor *CXCR4* during primordial germ cell migration in the chick embryo (Stebler *et al*., 2004). We now report a more general expression analysis of *SDF-1* during chick embryo development, pointing towards its functional relevance to migratory cell populations. Our *SDF-1* RNA probe is designed to bind with *SDF-1*α mRNA. However as there is considerable homology between the 5'end of the *SDF-1*α and *SDF-1*β, it is difficult to discriminate between the expression of *SDF-1*α and *SDF-1*β due to the small splice specific probe lengths. Our probe therefore was able to detect the expression of both *SDF-1*α and *SDF-1*β.

In the developing embryo, *CXCR4* has been described as the most abundantly expressed chemokine receptor, the expression starting as early as gastrulation stages (McGrath *et a*l., 1999). We have previously reported the expression pattern of *CXCR4* in the developing chick embryo (Yusuf *et al*., 2005). In our present work, we noticed that complementarity exists between the expression pattern of *SDF-1* and *CXCR4* during several stages of embryonic development. This complementarity has also been reported by others in mouse and zebrafish embryos (McGrath *et al*., 1999; Doitsidou *et al*., 2002; Vasyutina *et al*., 2005).

As in the case of the mouse embryos, the expression domains of *SDF-1* and *CXCR4* in the chick embryos also undergo a dramatic profile change along the antero-posterior axis as development proceeds. At the start of gastrulation, *SDF-1* transcripts can be detected in the epiblast, whereas at these stages, the expression of *CXCR4* although also present in the epiblast, is mainly restricted to the invaginating mesoderm and endoderm at the level of the primitive streak. At around stage HH6, the mesodermal signal is more prominent as reported previously (Stebler *et al*., 2004). The expression domains of *CXCR4* and *SDF-1* in the gastrulating tissue point towards a probable autocrine interaction between the ligand and receptor that guide the migrating epiblast cells. Similar autocrine interactions between the two have been described during endothelial cell branching (Salvucci *et al*., 2002). Once the three germ layers have been established and the mesoderm starts to organize itself into paraxial, intermediate and lateral plate mesoderm at HH14-16, the ectodermal expression of *SDF-1* is maintained above the differentiating mesoderm, which at this stage is positive for *CXCR4* (McGrath *et al*., 1999; Yusuf *et al*., 2005). However, as the paraxial mesoderm organizes to form epithelial somites, there is a progressive decrease of *SDF-1* transcripts cranially in the overlying ectoderm. As the *CXCR4/SDF-1* axis has been implicated in cell migration, it is probable that receptor-ligand interactions are needed for the cell movements that are occurring as the paraxial mesoderm arranges to give rise to epithelial somites. Similar interactions may also be active in the maturation of the kidney apparatus which develops from the intermediate mesoderm that expresses *CXCR4* (Yusuf *et al*., 2005).

A shift of expression domain from ectodermal to mesenchymal tissue similar to the one described in murine embryos was also observed in chick embryos (McGrath *et al*., 1999). From stage HH18 onwards, there are no *SDF-1* transcripts to be seen in the overlying ectoderm and its derivative, the neural tube. *SDF-1* expression is now evident in the sclerotome and mesenchymal tissue surrounding the aorta. At similar stages the expression profile of *CXCR4* has now shifted to the neural tube as described before (Yusuf *et al*., 2005; McGrath *et al*., 1999). The role of *SDF-1* has been well investigated in endothelial cell organization and vessel formation (Chen *et al*., 2007). Recently *SDF-1* has also been advocated as a probable candidate for therapeutic neovascularization (Zhou *et al*., 2007). *SDF-1* transcripts could also be detected in the lateral plate mesoderm and the genital ridge (data not shown) as published earlier (Stebler *et al*., 2004).

SDF-1 was detected at sites of active vessel formation in the intersomitic regions and the interdigital zones. We had earlier described streaks of *CXCR4* positive cells moving into the limbs from the intersomitic regions at stage HH19-20 (Yusuf *et al*., 2005), at similar stages we also observe a faint *SDF-1* staining in the limb mesenchyme in Fig1 I and L. The *SDF-1/CXCR4* axis has been shown to induce chemotaxis and cell migration in several physiological and pathological situations during development and disease (Murdoch 2000). In context of the embryo, *SDF-1* probably functions in hand with other chemoattractants like Scatter Factor/Hepatocyte Growth Factor (*SF/HGF*) to make possible the migration of the myogenic and angiogenic precursors into the limb mesenchyme. Recently, work from the lab of Carmen Birchmeier and from our lab has implicated *SDF-1* signalling in muscle patterning (Vasyutina *et al*., 2005; Yusuf *et al*., 2006).

SDF-1 expression was also observed in the dorsal aspect of the forelimb and cloacal region (data not shown) at HH 26-27, pointing towards a possible role in pectoral gridle and cloacal muscle formation.

Our expression analysis shows that *SDF-1* has considerable complementarity to the expression pattern of *CXCR4* in chick embryos. Furthermore, its expression pattern is highly suggestive for its role in cell migration and vasculogenesis during embryogenesis and organogenesis.

Materials and Methods

Preparation of probe and in situ *hybridization*

Fertilized chicken eggs obtained from a local breeder were incubated at 38°C and 80% humidity. Embryos were staged according to Hamburger and Hamilton (1995). After removing the extra-embryonic membrane, embryos were fixed overnight in 4% PFA at 4°C. For dehydration, embryos were entered into a series of different graded of methanol and were subsequently stored at -20°C or directly entered into the procedure of *in situ* hybridization (Nieto, *et al.,* 1996) with SDF-1 antisense RNA probe, which was generated in this study. For construction of SDF-1 plasmid, the chick *SDF-1*α gene was obtained by using RT-PCR with a

pair of *SDF-1α* gene specific primers (sense primer: 5' GCCTGCACCGTCGCCAGAATG 3'; and antisense primer: 5' AGGCCAACTCCAAACCCATCTTCA 3'). The RT-PCR product (425bp) was cloned into pDrive vector and confirmed by sequencing. For preparation of the RNA antisense probe to detect *SDF-1* gene expression patterns in this study, the plasmid was linearized with Not I and synthesized with T7 RNA polymerase. The sense RNA probe for SDF-1 was synthesized as control. After the *in situ* hybridization experiment, the embryos were analyzed and photographed. The embryos were further sectioned by using a Leica vibratome at a thickness of 35-60μm. For permanent slides, sections were embedded in Aquatex from Merck.

Acknowledgements

All authors would like to extend their thanks to Ellen Gimbel, Susanna Konradi and Ulrike Pein for their excellent technical assistances. We acknowledge the support of "molecular mechanisms of migration, invasion and metastasis", a Baden-Württemberg grant, the Myores Project (511978) funded by the EU's sixth framework Programme and Grako 1104.

References

ARA, T., NAKAMURA, Y., EGAWA, T., SUGIYAMA, T., ABE, K., KISHIMOTO, T., MATSUI, Y. and NAGASAWA, T. (2003). Impaired colonization of the gonads by primordial germ cells in mice lacking a chemokine, stromal cell-derived factor-1 (sdf-1). *Proc Natl Acad Sci USA* 100: 5319-23.

BAGRI, A., GURNEY, T., HE, X., ZOU, Y.R., LITTMAN, D.R., TESSIER-LAVIGNE, M. and PLEASURE, S.J. (2002). The chemokine sdf1 regulates migration of dentate granule cells. *Development* 129: 4249-60.

BALABANIAN, K., LAGANE, B., INFANTINO, S., CHOW, K.Y., HARRIAGUE, J., MOEPPS, B., ARENZANA-SEISDEDOS, F., THELEN, M. and BACHELERIE, F. (2005). The chemokine sdf-1/cxcl12 binds to and signals through the orphan receptor rdc1 in t lymphocytes. *J Biol Chem* 280: 35760-6.

BALKWILL, F. (2004). The significance of cancer cell expression of the chemokine receptor cxcr4. *Semin Cancer Biol* 14: 171-9.

BLEUL, C.C., FUHLBRIGGE, R.C., CASASNOVAS, J.M., AIUTI, A. and SPRINGER, T.A. (1996). A highly efficacious lymphocyte chemoattractant, stromal cell-derived factor 1 (sdf-1). *J Exp Med* 184: 1101-9.

CHALASANI, S.H., SABELKO, K.A., SUNSHINE, M.J., LITTMAN, D.R. and RAPER, J.A. (2003). A chemokine, sdf-1, reduces the effectiveness of multiple axonal repellents and is required for normal axon pathfinding. *J Neurosci* 23: 1360-71.

CHEN, T., BAI, H., SHAO, Y., ARZIGIAN, M., JANZEN, V., ATTAR, E., XIE, Y., SCADDEN, D.T. and WANG, Z.Z. (2007). Stromal cell-derived factor-1/cxcr4 signaling modifies the capillary-like organization of human embryonic stem cell-derived endothelium in vitro. *Stem Cells* 25: 392-401.

DAMBLY-CHAUDIERE, C., CUBEDO, N. and GHYSEN, A. (2007). Control of cell migration in the development of the posterior lateral line: Antagonistic interactions between the chemokine receptors cxcr4 and cxcr7/rdc1. *BMC Dev Biol* 7: 23.

DOITSIDOU, M., REICHMAN-FRIED, M., STEBLER, J., KOPRUNNER, M., DORRIES, J., MEYER, D., ESGUERRA, C.V., LEUNG, T. and RAZ, E. (2002). Guidance of primordial germ cell migration by the chemokine sdf-1. *Cell* 111: 647-59.

DUNUSSI-JOANNOPOULOS, K., ZUBEREK, K., RUNYON, K., HAWLEY, R.G., WONG, A., ERICKSON, J., HERRMANN, S. and LEONARD, J.P. (2002). Efficacious immunomodulatory activity of the chemokine stromal cell-derived factor 1 (sdf-1): Local secretion of sdf-1 at the tumor site serves as t-cell chemoattractant and mediates t-cell-dependent antitumor responses. *Blood* 100: 1551-8.

HONCZARENKO, M., LE, Y., GLODEK, A.M., MAJKA, M., CAMPBELL, J.J., RATAJCZAK, M.Z. and SILBERSTEIN, L.E. (2002). Ccr5-binding chemokines modulate cxcl12 (sdf-1)-induced responses of progenitor b cells in human bone marrow through heterologous desensitization of the cxcr4 chemokine receptor. *Blood* 100: 2321-9.

HORI, M., MITSUHASHI, S., KOBAYASHI, T., NAGASAWA, T., MORI, N., MIWA, M. and ABE, T. (1994). Cd30-positive lymphoma in human t-cell leukaemia virus type 1 carrier without monoclonal integration of htlv-1. *Br J Haematol* 88: 419-20.

HOSHI, M., NAGASAWA, T., ABE, T., SATOH, T. and MITSUI, Y. (1994). Maturation of human megakaryocytic cell line (cmk-7) on the human umbilical vein endothelial cell (ec) monolayer. *Int J Hematol* 59: 191-9.

IWASAKI, Y., NIWA, S., NAKANO, H., NAGASAWA, T. and YAMANE, T. (1994). Purification and properties of phosphatidylinositol-specific phospholipase c from streptomyces antibioticus. *Biochim Biophys Acta* 1214: 221-8.

KIJIMA, T., MAULIK, G., MA, P.C., TIBALDI, E.V., TURNER, R.E., ROLLINS, B., SATTLER, M., JOHNSON, B.E. and SALGIA, R. (2002). Regulation of cellular proliferation, cytoskeletal function and signal transduction through cxcr4 and c-kit in small cell lung cancer cells. *Cancer Res* 62: 6304-11.

KIM, C.H. and BROXMEYER, H.E. (1999). Chemokines: Signal lamps for trafficking of t and b cells for development and effector function. *J Leukoc Biol* 65: 6-15.

KNAUT, H., WERZ, C., GEISLER, R. and NUSSLEIN-VOLHARD, C. (2003). A zebrafish homologue of the chemokine receptor cxcr4 is a germ-cell guidance receptor. *Nature* 421: 279-82.

KOJIMA, H., SUZUKAWA, K., YATABE, Y., HORI, M., NAGASAWA, T. and ABE, T. (1994). Establishment of a new natural killer (nk) cell line, tks-1, from a patient with aggressive type of large granular lymphocyte (lgl) leukemia. *Leukemia* 8: 1999-2004.

KOSHIBA, T., HOSOTANI, R., MIYAMOTO, Y., IDA, J., TSUJI, S., NAKAJIMA, S., KAWAGUCHI, M., KOBAYASHI, H., DOI, R., HORI, T. *et al.* (2000). Expression of stromal cell-derived factor 1 and cxcr4 ligand receptor system in pancreatic cancer: A possible role for tumor progression. *Clin Cancer Res* 6: 3530-5.

LAPIDOT, T. and KOLLET, O. (2002). The essential roles of the chemokine sdf-1 and its receptor cxcr4 in human stem cell homing and repopulation of transplanted immune-deficient nod/scid and nod/scid/b2m(null) mice. *Leukemia* 16: 1992-2003.

LU, M., GROVE, E.A. and MILLER, R.J. (2002). Abnormal development of the hippocampal dentate gyrus in mice lacking the cxcr4 chemokine receptor. *Proc Natl Acad Sci USA* 99: 7090-5.

LUSTER, A.D. (1998). Chemokines—chemotactic cytokines that mediate inflammation. *N Engl J Med* 338: 436-45.

MCGRATH, K.E., KONISKI, A.D., MALTBY, K.M., MCGANN, J.K. and PALIS, J. (1999). Embryonic expression and function of the chemokine sdf-1 and its receptor, cxcr4. *Dev Biol* 213: 442-56.

MULLER, W.J. and NEVILLE, M.C. (2001). Introduction: Signaling in mammary development and tumorigenesis. *J Mammary Gland Biol Neoplasia* 6: 1-5.

MURDOCH, C. (2000). Cxcr4: Chemokine receptor extraordinaire. *Immunol Rev* 177: 175-84.

NAGASAWA, T., KIKUTANI, H. and KISHIMOTO, T. (1994). Molecular cloning and structure of a pre-b-cell growth-stimulating factor. *Proc Natl Acad Sci USA* 91: 2305-9.

NAGASAWA, T., TACHIBANA, K. and KISHIMOTO, T. (1998). A novel cxc chemokine pbsf/sdf-1 and its receptor cxcr4: Their functions in development, hematopoiesis and hiv infection. *Semin Immunol* 10: 179-85.

NIETO, M.A., PATEL, K. and WILKINSON, D.G. (1996). In situ hybridization analysis of chick embryos in whole mount and tissue sections. *Methods Cell Biol* 51: 219-35.

PUJOL, F., KITABGI, P. and BOUDIN, H. (2005). The chemokine sdf-1 differentially regulates axonal elongation and branching in hippocampal neurons. *J Cell Sci* 118: 1071-80.

RATAJCZAK, M.Z., ZUBA-SURMA, E., KUCIA, M., RECA, R., WOJAKOWSKI, W. and RATAJCZAK, J. (2006). The pleiotropic effects of the sdf-1-cxcr4 axis in organogenesis, regeneration and tumorigenesis. *Leukemia* 20: 1915-24.

SALVUCCI, O., YAO, L., VILLALBA, S., SAJEWICZ, A., PITTALUGA, S. and TOSATO, G. (2002). Regulation of endothelial cell branching morphogenesis by endogenous chemokine stromal-derived factor-1. *Blood* 99: 2703-11.

SCHRADER, A.J., LECHNER, O., TEMPLIN, M., DITTMAR, K.E., MACHTENS, S., MENGEL, M., PROBST-KEPPER, M., FRANZKE, A., WOLLENSAK, T., GATZLAFF, P. *et al.* (2002). Cxcr4/cxcl12 expression and signalling in kidney cancer. *Br J Cancer* 86: 1250-6.

SCOTTON, C., MILLIKEN, D., WILSON, J., RAJU, S. and BALKWILL, F. (2001). Analysis of cc chemokine and chemokine receptor expression in solid ovarian

tumours. *Br J Cancer* 85: 891-7.

SCOTTON, C.J., WILSON, J.L., MILLIKEN, D., STAMP, G. and BALKWILL, F.R. (2001). Epithelial cancer cell migration: A role for chemokine receptors? *Cancer Res* 61: 4961-5.

SCOTTON, C.J., WILSON, J.L., SCOTT, K., STAMP, G., WILBANKS, G.D., FRICKER, S., BRIDGER, G. and BALKWILL, F.R. (2002). Multiple actions of the chemokine cxcl12 on epithelial tumor cells in human ovarian cancer. *Cancer Res* 62: 5930-8.

SHIROZU, M., NAKANO, T., INAZAWA, J., TASHIRO, K., TADA, H., SHINOHARA, T. and HONJO, T. (1995). Structure and chromosomal localization of the human stromal cell-derived factor 1 (sdf1) gene. *Genomics* 28: 495-500.

STEBLER, J., SPIELER, D., SLANCHEV, K., MOLYNEAUX, K.A., RICHTER, U., COJOCARU, V., TARABYKIN, V., WYLIE, C., KESSEL, M. and RAZ, E. (2004). Primordial germ cell migration in the chick and mouse embryo: The role of the chemokine sdf-1/cxcl12. *Dev Biol* 272: 351-61.

STUMM, R.K., RUMMEL, J., JUNKER, V., CULMSEE, C., PFEIFFER, M., KRIEGLSTEIN, J., HOLLT, V. and SCHULZ, S. (2002). A dual role for the sdf-1/cxcr4 chemokine receptor system in adult brain: Isoform-selective regulation of sdf-1 expression modulates cxcr4-dependent neuronal plasticity and cerebral leukocyte recruitment after focal ischemia. *J Neurosci* 22: 5865-78.

TAICHMAN, R.S., COOPER, C., KELLER, E.T., PIENTA, K.J., TAICHMAN, N.S. and MCCAULEY, L.K. (2002). Use of the stromal cell-derived factor-1/cxcr4 pathway in prostate cancer metastasis to bone. *Cancer Res* 62: 1832-7.

VASYUTINA, E., STEBLER, J., BRAND-SABERI, B., SCHULZ, S., RAZ, E. and BIRCHMEIER, C. (2005). Cxcr4 and gab1 cooperate to control the development of migrating muscle progenitor cells. *Genes Dev* 19: 2187-98.

XIANG, Y., LI, Y., ZHANG, Z., CUI, K., WANG, S., YUAN, X.B., WU, C.P., POO, M.M. and DUAN, S. (2002). Nerve growth cone guidance mediated by g protein-coupled receptors. *Nat Neurosci* 5: 843-8.

YUN, H.J. and JO, D.Y. (2003). Production of stromal cell-derived factor-1 (sdf-1)and expression of cxcr4 in human bone marrow endothelial cells. *J Korean Med Sci* 18: 679-85.

YUSUF, F., REHIMI, R., DAI, F. and BRAND-SABERI, B. (2005). Expression of chemokine receptor cxcr4 during chick embryo development. *Anat Embryol (Berl)* 210: 35-41.

YUSUF, F., REHIMI, R., MOROSAN-PUOPOLO, G., DAI, F., ZHANG, X. and BRAND-SABERI, B. (2006). Inhibitors of cxcr4 affect the migration and fate of cxcr4+ progenitors in the developing limb of chick embryos. *Dev Dyn* 235: 3007-15.

ZHOU, B., HAN, Z.C., POON, M.C. and PU, W. (2007). Mesenchymal stem/stromal cells (msc) transfected with stromal derived factor 1 (sdf-1) for therapeutic neovascularization: Enhancement of cell recruitment and entrapment. *Med Hypotheses* 68: 1268-71.

ZHU, Y., YU, T., ZHANG, X.C., NAGASAWA, T., WU, J.Y. and RAO, Y. (2002). Role of the chemokine sdf-1 as the meningeal attractant for embryonic cerebellar neurons. *Nat Neurosci* 5: 719-20.

ZOU, Y.R., KOTTMANN, A.H., KURODA, M., TANIUCHI, I. and LITTMAN, D.R. (1998). Function of the chemokine receptor cxcr4 in haematopoiesis and in cerebellar development. *Nature* 393: 595-9.

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