Expression and function of cell adhesion molecules during neural crest migration

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Abstract

Neural crest cells are highly migratory cells that give rise to many derivatives including peripheral ganglia, craniofacial structures and melanocytes. Neural crest cells migrate along defined pathways to their target sites, interacting with each other and their environment as they migrate. Cell adhesion molecules are critical during this process. In this review we discuss the expression and function of cell adhesion molecules during the process of neural crest migration, in particular cadherins, integrins, members of the immunoglobulin superfamily of cell adhesion molecules, and the proteolytic enzymes that cleave these cell adhesion molecules. The expression and function of these cell adhesion molecules and proteases are compared across neural crest emigrating from different axial levels, and across different species of vertebrates.

Keywords

Neural crest, cell adhesion, migration, mouse, chick, Xenopus
Introduction

Neural crest (NC) cells migrate extensively before giving rise to a variety of cell types. NC cell-cell and cell-matrix interactions are an essential part of the migration process. In fact, NC cell migration depends on optimal levels of adhesion, as too little or too much adhesion result in migration defects (Anderson et al., 2006b). In this review, we describe how the immunoglobulin superfamily of cell adhesion molecules (IgCAMs), cadherins and integrins, and the proteolytic enzymes that cleave them, regulate the migration of NC cells arising from different regions of the neural axis.

NC cells form at the junction of the neural plate and epidermal ectoderm during neurulation. The formation of the NC requires multiple signaling pathways and transcription factors to establish a pool of pre-migratory neural crest cells at the dorsal most regions of the neural folds (Betancur et al., 2010). Once NC cells have formed, they undergo an epithelial-mesenchymal transition (EMT) in order to migrate away from the neural tube. They begin migration from the dorsal part of the neural tube prior to, during or after the closure of the neural tube, depending on the axial level and species. Cell adhesion molecules play roles in NC EMT, migration and also in their aggregation at their final destination, but in this review we focus primarily on the expression and function of cell adhesion molecules during NC migration. Changes in cell-cell adhesion, cell-matrix adhesion and the cell cytoskeleton during EMT have been reviewed recently (Clay and Halloran, 2011; Taneyhill, 2008; Thiery et al., 2009).

NC cells from different anteroposterior axial levels follow different migratory pathways and give rise to different derivatives (Le Douarin and Kalcheim, 1999; Fig. 1). The main sub-divisions of the neural crest are cranial, vagal and trunk. In this review, we discuss the role of cell adhesion molecules in regulating the migration of each of these NC populations. The expression and function of the main cell adhesion molecules are summarized in Table 1.
**Cranial Neural Crest**

Cranial NC cells give rise to much of the cartilage and bone of the skull and face, as well as other connective tissues, and contribute to neurons and glia in cranial ganglia. The cranial NC arise from the midbrain to somite 5 and migrate underneath the ectoderm in a ventral direction to populate the frontonasal process, branchial arches, and the trigeminal and epibranchial ganglia. While there is some intermingling of NC cells arising from different anteroposterior levels (Kulesa and Fraser, 1998), NC cells from particular axial levels follow pathways to populate corresponding facial processes (Lumsden et al., 1991; Sadaghiani and Thiebaud, 1987; Trainor et al., 2002). Particular cranial regions express inhibitory molecules and repel NC cells, narrowing the migratory pathways of NC from certain axial levels (Farlie et al., 1999; Golding et al., 2004). The earliest migrating cells populate the facial processes and give rise to mesenchymal derivatives, while later migrating NC cells remain in more dorsal regions and contribute to cranial ganglia (Baker et al., 1997).

In contrast to other vertebrate species, the initial migration of cranial NC in *Xenopus* is a collective migration, followed by a wave of single cell migration (Alfandari et al., 2003). This collective migration process involves contact inhibition of locomotion in which protrusions of NC cells collapse when they contact another NC cell (Carmona-Fontaine et al., 2008), and collective chemotaxis, mediated by the chemokine SDF1 (Theveneau et al., 2010). In other vertebrates, such as such as chick, time-lapse imaging revealed that many NC cells migrate to the branchial arches in dynamic chains (Kulesa and Fraser, 1998; 2000). Within these chains, the NC cells are in nearly constant contact with each other over short and long distances via lamellipodia or filopodia (Teddy and Kulesa, 2004). Moreover, some migrating cranial NC exchange cytoplasmic material through thin cellular bridges (McKinney et al., 2011). Cell-cell adhesion is involved in each of these modes of migration, and while N-cadherin has been implicated in contact inhibition of locomotion in *Xenopus*, the molecular mechanisms involved in chain migration have not yet been identified (Alfandari et al., 2010; Teddy and Kulesa, 2004; Theveneau et al., 2010).
Cadherins

The cadherin superfamily is divided into subfamilies, and of these, type I and type II classical cadherins, and also protocadherins have been implicated in NC development and migration. These cadherins are transmembrane molecules that extracellularly undergo primarily homophilic binding to mediate cell-cell adhesion (Gumbiner, 2005). The strength of the cell-cell adhesion differs between cadherins: type I cadherins promote stronger adhesiveness than type II cadherins (Chu et al., 2006). In addition, some cadherins are also able to bind other molecules extracellularly (Gumbiner, 2005). Intracellularly, classical cadherins interact with catenins, while protocadherins can interact with Fyn kinases, each of which have signaling roles and various effects on cell behavior (Gumbiner, 2005). The role of cadherins in mediating cell adhesion is complex and can be regulated at many levels, both intracellularly and extracellularly (Halbleib and Nelson, 2006). Cadherins are involved in NC EMT, migration of cranial NC and the aggregation of cranial ganglia.

Cadherin 6B, a type II cadherin, is expressed by avian premigratory NC (Nakagawa and Takeichi, 1995), and is transcriptionally downregulated by Snail2 prior to emigration (Taneyhill et al., 2007). Cadherin-6B plays a role in the EMT undertaken as NC begin to migrate (Coles et al., 2007; Taneyhill, 2008). Inhibition by morpholino of cadherin-6B in chick embryos in ovo promoted premature and increased migration of the cranial NC, while overexpression reduced the number of migrating cranial NC and increased ectopic NC in the neural tube lumen in vivo, but not in vitro (Coles et al., 2007). These findings are different from the effect of manipulation of cadherin-6B in trunk NC (see below).

Expression of the type I N-cadherin by migrating avian cranial NC in vivo has not been detected using antibody labeling (Bronner-Fraser et al., 1992b; Duband et al., 1988), however, it has been detected by qPCR (McLennan et al., 2012). A possible explanation for this discrepancy is that processing for immunofluorescence may only allow visualization of N-cadherin associated with adherens junctions, and N-cadherin in migrating NC in vitro is mostly soluble and not associated with cell-cell contacts (Monier-Gavelle and Duband, 1995). In contrast, during early cranial NC migration in Xenopus embryos N-cadherin mRNA and protein can be detected (Theveneau et
al., 2010), and both overexpression and morpholino knockdown of N-cadherin in vivo block cranial NC emigration (Theveneau et al., 2010). In vitro studies demonstrated a role for N-cadherin during cranial NC collective migration in *Xenopus*. Function blocking antibodies to N-cadherin prevented contact inhibition of locomotion and collective polarization of cranial NC cells toward an SDF1 gradient (Theveneau et al., 2010). Interestingly, cranial NC cells treated with an N-cadherin morpholino were highly motile in culture, and dispersed more rapidly than control cells (Theveneau et al., 2010). This behaviour is similar to migratory cardiac NC from N-cadherin null mutant mice, which have increased speed and persistence of movement in vitro, but decreased directionality (Xu et al., 2001). A model of *Xenopus* cranial NC migration has been proposed, in which N-cadherin-mediated cell adhesion causes contact-dependent cell polarity, and when combined with a chemotactic signal, allows collective chemotaxis of cranial NC (Theveneau et al., 2010). In zebrafish, intracellular trafficking of N-cadherin to the cell membrane is regulated in a subset of cranial NC by the Wnt target gene, Ovo1, most likely through activity of Rab proteins (Piloto and Schilling, 2010). However loss of functional N-cadherin in the zebrafish pac mutant does not appear to perturb migration of cranial NC (Lele et al., 2002), and the impact of loss of function of N-cadherin in migrating NC in vivo in *Xenopus* is not yet known.

Cadherin 11, a type II cadherin, is expressed by migrating cranial NC in mouse (Kimura et al., 1995) and *Xenopus* (Hadeball et al., 1998; Vallin et al., 1998). Mice homozygous for a mutant non-adhesive cadherin 11 have shorter frontal bones, but no reported cranial NC migration defects (Manabe et al., 2000), whereas inhibition of cadherin 11 in *Xenopus* in vivo revealed an important role in cranial NC migration (Borchers et al., 2001; Kashef et al., 2009). Knockdown of cadherin 11 inhibits NC migration into the second and more caudal branchial arches only, and mutant cells do not form filopodia or lamellipodia (Kashef et al., 2009). In vivo rescue with mutated versions of cadherin 11 showed that expression of the cytoplasmic tail is sufficient for *Xenopus* cranial NC migration as long as it is linked to the plasma membrane. The β-catenin binding site and a binding site for the guanine exchange factor Trio are critical intracellular domains; as over-expression of human cadherin 11, Trio or constitutively active small Rho GTPases rescued the loss of cadherin 11 in vivo, whereas over-
expression of mouse cadherin 6 was unable to rescue migration (Kashef et al., 2009). Furthermore, cleavage of the extracellular domain of \textit{Xenopus} cadherin 11 is necessary for cranial NC migration (see below). Thus cadherin-11 appears to act together with \(\beta\)-catenin and Trio at the plasma membrane to initiate protrusive activity and drive cranial NC migration through the activation of small Rho GTPases. Interestingly, Trio can have multiple cell surface protein partners (Backer et al., 2007; Briancon-Marjollet et al., 2008; Yano et al., 2011), but whether it normally binds to partners other than cadherin 11 in \textit{Xenopus} cranial NC is not known. \textit{Xenopus} cranial NC also express a proto-cadherin named PCNS (Rangarajan et al., 2006). Knockdown of PCNS by morpholino inhibits cranial NC migration \textit{in vivo} and the cells also fail to migrate and instead disaggregate when cultured on fibronectin (Rangarajan et al., 2006). The exact function of PCNS in cranial NC migration remains to be determined, however it has been suggested that PCNS may influence planar cell polarity signalling, which is required for contact inhibition of locomotion in \textit{Xenopus} cranial NC migration (Alfandari et al., 2010).

Avian cadherin 7, murine cadherin 6 and rat cadherin 19 are related type II cadherins that are expressed by migrating cranial NC (Inoue et al., 1997; Nakagawa and Takeichi, 1995; Takahashi and Osumi, 2005); however, a functional role has not yet been established, and mice null for cadherin 6 are viable with no reported cranial NC defects (Mah et al., 2000). Cadherin 7 expression has recently been shown to be expressed at higher levels in trailing avian migratory cranial NC than in the leading migratory NC \textit{in vivo} (McLennan et al., 2012), suggesting cell-cell adhesion may be more important for following rather than leading cells. Using transfected cell lines, cadherin 7 has been shown to have higher adhesivity compared to cadherin 11, and both type II cadherins have much lower adhesivity compared to N-cadherin (Chu et al., 2006).

N-cadherin is expressed by chick placodal cells but not cranial NC during the aggregation of cranial sensory ganglia (Shiau and Bronner-Fraser, 2009). Blocking N-cadherin translation in trigeminal placode cells with morpholinos \textit{in ovo} led to misshapen ganglia and decreased aggregation of the placode cells (Shiau and Bronner-Fraser, 2009). The cell-surface expression of N-cadherin on placode cells
was regulated post-translationally by cranial NC-mediated Slit1 activation of Robo2 expressed by placode cells (Shiau and Bronner-Fraser, 2009).

**Integrins**

Integrins are heterodimeric transmembrane receptors composed of alpha and beta subunits, which bind extracellularly to a variety of extracellular matrix molecules and other ligands, depending on the combination of subunits (van der Flier and Sonnenberg, 2001). Integrins are regulated on many levels; their affinity for ligands can be reversibly altered by conformational changes, which can be mediated by intracellular or extracellular factors (Hynes, 2002; Luo et al., 2007). Trafficking of integrins and distribution of integrins at the cell membrane can also affect the level of cell adhesion (Margadant et al., 2011; Ulrich and Heisenberg, 2009). Furthermore, integrin-ligand interactions can result in intracellular changes including activation of signal transduction pathways, regulating survival, proliferation and gene expression (Hynes, 2002; Margadant et al., 2011).

Cranial NC express a variety of integrins, some of which are restricted to specific axial levels, and some to particular species. The integrins that are expressed by migrating cranial NC in multiple species are the β1 (Bronner-Fraser, 1985; Gawantka et al., 1992; Krotoski and Bronner-Fraser, 1986; Pietri et al., 2004; Ransom et al., 1993) and α5 subunits (Alfandari et al., 2003; Bajanca et al., 2004; Joos et al., 1995; Wang and Burke, 1995). Integrin α5β1 mediates migration of cranial NC from multiple species on fibronectin in vitro (Alfandari et al., 2003; Kil et al., 1998; Lallier and Bronner-Fraser, 1992). Inhibiting the function of β1 integrins in chick in ovo resulted in reduced cranial NC migration and an ectopic buildup of NC cells in the neural tube (Boucaut et al., 1984; Bronner-Fraser, 1985; 1986). Surprisingly, mouse conditional knockout studies of β1 integrin using the Wnt1-Cre line showed migration of cranial NC cells to the branchial arches (Turlo et al., 2012), however levels of proliferation and apoptosis were not assessed. Moreover, in mice null for fibronectin, cranial NC cells also still migrate to the branchial arches, but the number of cranial NC cells is reduced due to increased apoptosis and decreased proliferation (Mittal et
Similarly, in integrin α5 null mice, apoptosis occurs in NC cells migrating to the second branchial arch (Goh et al., 1997; Mittal et al., 2010). This suggests that fibronectin and integrin α5 are critical for the survival of cranial NC, rather than migration. Furthermore, the restricted regional effect of loss of α5 suggests that there is compensation by other integrins rostral to the second arch. Indeed, integrin α4 is a candidate whose expression is restricted to NC rostral to the second branchial arch (Pinco et al., 2001). Exposure to α4 function-blocking antibodies in mouse cultures inhibited cranial NC migration (Kil et al., 1998), however at least some cranial NC migration is normal in α4 null mice, as the trigeminal ganglia are present (Yang et al., 1995). In contrast to the axially restricted expression of α4 in mouse, chick cranial NC express α4 at all axial levels (Kil et al., 1998), while Xenopus cranial NC do not appear to express α4 (Whittaker and Desimone, 1998). The expression of β3, α6 and αv by cranial NC also shows species differences (Alfandari et al., 2003; Joos et al., 1998; Lallier et al., 1996; Pietri et al., 2003; Ransom et al., 1993; Wang and Burke, 1995), but the significance of these differences is not yet clear.

While it may be surprising that loss of fibronectin or β1 integrin in mice does not appear to prevent cranial NC migration, many other extracellular matrix molecules are present within the cranial pathway. Chick cranial and trunk NC regulated surface integrin expression, location and activity in different ways in response to different concentrations and types of extracellular matrix substrates in vitro. On high concentrations of laminin, cranial NC migrated faster than trunk NC (Strachan and Condic, 2003), by modulating surface integrin expression in a manner involving integrin receptor recycling (Strachan and Condic, 2004). In contrast, on fibronectin, receptor recycling did not occur (Strachan and Condic, 2004). Cranial NC reduced surface expression of α4 integrin on high concentrations of fibronectin, whereas trunk NC did not (Strachan and Condic, 2003). Migration on fibronectin was regulated by the activation of integrins, and interestingly cranial and trunk NC responded to integrin activation in different ways depending on the concentration of the fibronectin substrate (Strachan and Condic, 2008). Integrin activation of cranial NC on low concentrations of fibronectin increased avidity (integrin clustering), and on high concentrations of fibronectin increased affinity (Strachan and Condic, 2008). In
contrast, in trunk NC, integrin activation on low concentrations of fibronectin increased affinity, and on high concentrations of fibronectin increased avidity. In each of these cases, integrin activation slowed migration on fibronectin, but not on laminin (Strachan and Condic, 2008). These differences highlight the complexity of integrin function and regulation, and demonstrate that the NC are capable not only of regulation of integrin activity in response to the extracellular environment, but also that NC at different axial levels have different responses and regulate integrin function and activity differently.

Matrix Metalloproteases

Matrix metalloproteases (MMPs and members of the ADAM family) cleave a variety of proteins, including extracellular matrix molecules, and cell surface molecules such as cadherins, integrins and IgCAMs (Edwards et al., 2008; VanSaun and Matrisian, 2006). The proteolysis of cell adhesion molecules can disrupt cell-cell adhesion and also release soluble extracellular or intracellular fragments that can have further signaling effects (Edwards et al., 2008; VanSaun and Matrisian, 2006). In addition, ADAMs contain a disintegrin loop which interacts with integrins to influence cell adhesion and migration (Huang et al., 2005), and the cytoplasmic domain of ADAMs can interact with a variety of proteins to affect catalytic activity, trafficking and cell signaling events (Edwards et al., 2008). MMPs and ADAMs have been implicated in regulating NC EMT and migration. ADAM13 and 19 are expressed in Xenopus cranial NC prior to migration, and early knockdown using morpholinos to ADAM13 (in Xenopus tropicalis, but not Xenopus laevis), or ADAM19, reduces the expression of cranial NC markers (Cousin et al., 2011; Neuner et al., 2009; Wei et al., 2010), and inhibits NC migration (Cousin et al., 2011; Neuner et al., 2009). Moreover, knocking down ADAM13 and 19 together inhibited NC migration to a greater extent than knockdown of either gene alone (Cousin et al., 2011), suggesting that although they have some distinct functions during NC development, there is also some compensation between these genes (Cousin et al., 2011; Wei et al., 2010).

ADAM13 continues to be expressed by cranial NC during migration in Xenopus (Alfandari et al., 1997) and is expressed in chick cranial NC pathways (Lin et al., 2007). ADAM33 is also expressed by migrating avian cranial NC (McLennan et al.,
and ADAM9 and ADAM11 by *Xenopus* cranial NC (Cai et al., 1998). Multiple ADAMs (ADAM10, 12, 15, 17, 19 and 33) are expressed by cranial NC or along the cranial NC migratory pathway in developing mouse embryos (Tomczuk, 2004). Blocking metalloprotease activity inhibited mouse cranial NC migration on fibronectin (Tomczuk, 2004); however the role of specific ADAMs or MMPs during mouse cranial NC migration is not yet known. Over-expression of full-length ADAM13 in *Xenopus* in vivo resulted in a large mass of NC cells near the neural tube (Alfandari et al., 2001). Expression of a protease defective ADAM13, which acts as a dominant negative, reduced migration of NC in the second and more caudal branchial arch streams (Alfandari et al., 2001), a similar phenotype to the knockdown of cadherin-11 (Kashef et al., 2009). Co-immunoprecipitation and Western blotting demonstrated that ADAM13 or ADAM9 cleaves cadherin-11, and inhibiting ADAM activity blocks cleavage of cadherin-11 in vivo (McCusker et al., 2009). Moreover, increased expression of ADAM13 can rescue the inhibition of NC migration caused by an over-expression of cadherin-11 (McCusker et al., 2009); demonstrating that ADAM13 mediated cleavage of cadherin-11 is required for normal cranial NC migration. Cleavage of the extracellular domain of cadherin-11 in *Xenopus* cranial NC by ADAM13 reduces cell-cell adhesion directly, and the cleaved domain is then able to bind to other full-length cadherin-11 molecules, further reducing cell-cell adhesion and promoting migration (McCusker et al., 2009). However, reducing cadherin-11 by morpholino is not sufficient alone to rescue a loss of ADAM13 in vivo (Cousin et al., 2012), demonstrating that ADAM13 has multiple functions in mediating the migration of cranial NC. ADAM13 can also cleave fibronectin, and by binding to fibronectin domains, promotes cell adhesion via β1 integrins (Gaultier et al., 2002). Manual separation of the cranial NC from the ectoderm and mesoderm in vivo partially rescues the loss of ADAM13 (Cousin et al., 2012), suggesting that cleavage or modification of extracellular matrix is important in allowing NC migration. Moreover, the loss of ADAM13 alone or both ADAM13 and ADAM19 together did not affect the migration of cranial NC in vitro. ADAM13 needs to be expressed by at least some cranial NC in order to allow normal migration of the population, and these data suggest that ADAM13 promotes migration partly by modifying the extracellular environment through which the cranial NC migrate (Cousin et al., 2012).
ADAM13 lacking the cytoplasmic domain is unable to rescue the inhibition of NC migration due to ADAM13 morpholino (Cousin et al., 2011). The cytoplasmic domain regulates function by both interacting with the adaptor protein PACSIN2, (Cousin et al., 2000) and by altering the subcellular localisation or activity of the protease domain after cleavage (Cousin et al., 2011). Cleavage of the cytoplasmic domain by γ-secretase during NC development allows the translocation of the cytoplasmic domain to the nucleus, altering the expression of multiple genes including Calpain8-a, a member of a protease family linked to cell migration (Cousin et al., 2011). Expression of both the extracellular domain of cadherin-11 combined with the cytoplasmic domain of ADAM13 is sufficient to rescue a loss of migration caused by combined ADAM13 and ADAM19 morpholino in vivo (Cousin et al., 2012). Therefore, ADAM13 promotes Xenopus cranial NC migration through multiple mechanisms; both extracellular cleavage of molecules, and regulation of transcription via its cytoplasmic domain.

Expression or function of ADAMs in particular subsets or stages of NC migration may influence the ability of NC cells to migrate to particular regions. For example, ADAM10 cleavage of a splice variant of the cell adhesion molecule CD44, CD44v6, appears to facilitate migration of NC into the cornea in chick embryos (Huh et al., 2007).

MMP9 is expressed by avian NC cells during EMT and migration, and also by some surrounding cells (Monsonego-Ornan et al., 2012). Inhibition of MMP9 activity reduces the number of cells emigrating from the neural tube at cranial levels, while overexpression, or MMP9 conditioned media, increases the number of migratory cranial NC in vitro and in vivo (Monsonego-Ornan et al., 2012). Thus MMP9 has a role in the onset of migration, possibly through degradation of the laminin basement membrane. Later inhibition of MMP9 in neural tube explants reduced the distance migrated by NC cells on the substrate, while MMP9 conditioned media significantly increased the number of emigrating cranial NC (Monsonego-Ornan et al., 2012). Migrating NC cells in these explants also demonstrated perturbations in the organization of stress fibres, and rapid loss of N-cadherin expression, suggesting N-
cadherin may also be a substrate of MMP9. In contrast to the phenotype in chick, MMP9 knockout mice do not display overt embryonic defects (Vu et al., 1998), perhaps due to redundancy with other members of the MMP family. MMP2, which is closely related to MMP9, is expressed by, or along, the cranial NC migratory pathway in chick, mouse and Xenopus, with some differences in expression between species (Cai et al., 2000; Robbins et al., 1999; Tomlinson et al., 2009). MMP2 in avian cranial NC is expressed at higher levels in leading compared to trailing cells (McLennan et al., 2012). In addition, MMP8 is expressed in mouse cranial NC (Giambernardi et al., 2001), and MMP14 in a subset of Xenopus NC migrating from rhombomeres 3 and 5 (Harrison et al., 2004). However the roles of these MMPs in cranial NC migration have not been determined.

IgCAMs

Members of the IgCAM family interact extracellularly either homophilically or with other proteins, and can exist as multiple isoforms due to alternative splicing. IgCAMs interact with numerous intracellular binding partners, ranging from effectors of signal-transduction pathways to cytoskeletal proteins. NCAM is detected on cranial NC cells in the chick prior to and in the early stages of migration (Bronner-Fraser et al., 1992b). NCAM mediated cell-cell adhesion is important for emigration and early stages of cranial NC migration, but is not necessary once migration is underway (Bronner-Fraser et al., 1992b). In Xenopus, NCAM is also expressed by cranial NC prior to migration, and later during aggregation of cranial ganglia, but is not expressed during NC migration (Balak et al., 1987).

Neuropilins and plexins can in some circumstances be involved in cell adhesion, however during NC migration they are best understood for their role in acting as receptors for the Ig domain containing Semaphorin molecules, which often act as chemorepellants or chemoattractants in the guidance of neural crest at multiple axial levels (reviewed in Schwarz and Ruhrberg, 2010). No other IgCAMs are known to play a role in the migration of cranial NC.
Vagal Neural Crest

Vagal NC cells, which arise adjacent to somites 1-7, include the most caudal cranial NC cells and the most rostral trunk NC cells. Vagal NC cells give rise to two distinct populations of cells, cardiac NC and enteric NC cells.

Cardiac NC Cells

Cardiac NC cells, which arise adjacent to somites 1-3, migrate beneath the ectoderm into branchial arches 3, 4, and 6, where they contribute to the great arteries, the aorticopulmonary septum, the conotruncal cushions in the outflow tract and cardiac ganglia of the developing heart (Kirby and Waldo, 1995; Waldo et al., 1998). Defects in cardiac NC migration result in severe cardiac defects involving outflow tract septation anomalies, such as persistent truncus arteriosus as well as various aortic arch anomalies (Kirby and Hutson, 2010). A number of cell adhesion molecules, including cadherins and integrins, as well as the proteolytic enzymes that cleave them, MMPs and ADAMs, play a role in cardiac NC migration and formation of the developing heart.

Cadherins

Cardiac NC cells express cadherin-6, cadherin-7 and cadherin-11, as they migrate to the heart. Avian cadherin-6B is expressed at the beginning of migration but is then rapidly down-regulated, while avian cadherin-7 and murine cadherin-6 and murine and *Xenopus* cadherin-11 are expressed throughout migration (Inoue et al., 1997; Kimura et al., 1995; Nakagawa and Takeichi, 1995; Vallin et al., 1998). Over-expression of these cadherins prevents NC emigration from the neural tube, while down regulation of cadherin-6B results in premature NC emigration (Coles et al., 2007; McCusker et al., 2009; Nakagawa and Takeichi, 1998).

The main cadherin involved in cardiac NC migration appears to be N-cadherin. Murine N-cadherin is weakly expressed during cardiac NC migration *in vitro* (Xu et al., 2001) and at regions of cell-cell contact by cardiac NC arriving at their destination *in vivo* (Luo et al., 2006). N-cadherin is dramatically up-regulated as the cells
condense in the outflow tract, and is required for the remodeling of the cardiac outflow tract (Luo et al., 2006). In the absence of N-cadherin, mouse cardiac NC cells migrate faster in vitro, but exhibit a loss of directionality resulting in an overall decrease in the distance migrated (Xu et al., 2001). Despite this, cardiac NC cells from N-cadherin deficient mice do reach the cardiac outflow tract, but are unable to undergo the normal morphogenetic changes, resulting in persistent truncus arteriosus, and in a thinning of the ventricular myocardium (Luo et al., 2006).

Connexin 43 (Cx43\(_{\alpha 1}\)) also plays a role in cardiac NC migration by interacting with N-cadherin (Xu et al., 2001) and mediating interactions with signaling pathways that regulate polarized cell movement (Xu et al., 2006). In N-cadherin deficient mice, cardiac NC cells display a reduced level of gap junction communication that is similar to Cx43\(_{\alpha 1}\) deficient NC cells (Xu et al., 2001). Cx43\(_{\alpha 1}\) deficient mice die at birth due to severe pulmonary outflow tract obstruction (Reaume et al., 1995). Loss of Cx43\(_{\alpha 1}\) inhibits cardiac NC cell migration, resulting in fewer cells reaching the outflow tract, while over-expression of Cx43\(_{\alpha 1}\) enhanced migration, resulting in more cardiac NC cells reaching the outflow tract (Huang et al., 1998). Like N-cadherin deficient cells, Cx43\(_{\alpha 1}\) deficient cardiac NC cells also show reduced directionality (Xu et al., 2001). Interactions between N-cadherin and Cx43\(_{\alpha 1}\) may be modulated through p120-catenin signaling (Xu et al., 2001). Although Cx43\(_{\alpha 1}\) forms gap junctions, cell-cell communication via gap junctions does not appear to modulate cardiac NC motility (Xu et al., 2006). Instead, Cx43\(_{\alpha 1}\) co-localises with many actin binding proteins and is hypothesized to play an important role in regulating the actin cytoskeleton during migration (Xu et al., 2006).

**Integrins**

Cardiac NC cells in Aves express multiple integrins, including the \(\alpha 4\) and \(\alpha 5\) subunits (Kil et al., 1998; Wang and Burke, 1995). The \(\alpha 5\) and \(\beta 1\) subunits have also been detected in mice (Goh et al., 1997; Xu et al., 2006), and murine cardiac NC synthesise fibronectin as they migrate (Mittal et al., 2010). In \(\alpha 5\) integrin null or conditional fibronectin null mutant mice, migration of cardiac NC cells to the heart still occurs (Goh et al., 1997; Mittal et al., 2010), but there is a reduction in the number of cardiac
NC and increased apoptosis, suggesting that, like more rostral cranial NC, α5 integrin and fibronectin are required for the proliferation of cardiac NC progenitors, and proliferation and survival of cardiac NC during migration (Mittal et al., 2010).

β1 integrins are up-regulated as avian cardiac NC cells emigrate from the neural tube \textit{in vivo} (Duband et al., 1986). Exposure to β1 integrin function blocking antibodies results in a reduction in the overall speed and directionality of mouse cardiac NC cells \textit{in vitro} (Xu et al., 2006). Although there are no detectable defects in cardiac NC migration in conditional Wnt1-Cre β1 integrin null mice, cardiac NC did not undergo vascular remodeling, resulting in embryonic lethality (Turlo et al., 2012). Similar cardiac defects and early death are also observed in mice where β1 integrins are eliminated prior to NC emigration from the neural tube (Pietri et al., 2004).

\textit{MMPs}

In order for cardiac NC cells to migrate through the extracellular matrix, they require the proteolytic activity of MMPs and/or ADAMs. Migrating avian cardiac NC cells express MMP2, MMP14 and tissue inhibitor of metalloproteinase (TIMP)-2 (Cantemir et al., 2004). Similarly to MMP2 at cranial levels, TIMP-2 is expressed at higher levels in leading compared to trailing cells (Cantemir et al., 2004). Use of antisense TIMP-2 oligonucleotides in chick resulted in perturbed cardiac NC formation and decreased NC cell migration away from the neural tube \textit{in ovo}, while overexpression of TIMP-2 increased cardiac neural cell motility \textit{in vitro} (Cantemir et al., 2004). TIMP-2 is required for activation of proMMP-2 by MMP14 (Cantemir et al., 2004). MMP-2 is expressed by avian cardiac NC \textit{in vitro} (Cantemir et al., 2004), and within the substrate through which cardiac NC cells migrate \textit{in vivo} (Cai et al., 2000). Pharmacological inhibition of MMP-2 in Aves decreases the distance cardiac NC cells migrate \textit{in vitro} and \textit{in vivo}, decreases motility and increases cell area and cell perimeter (Cai and Brauer, 2002). However, the migration of cardiac NC cells is not affected in MMP-2 or TIMP-2 null mutant mice (Caterina et al., 2000), suggesting that MMP-2 and TIMP-2 are either not involved in cardiac NC migration in mice \textit{in vivo}, or that there is functional redundancy with other matrix metalloproteases.
Cardiac NC cells also express ADAM19 in mice (Komatsu et al., 2007), ADAM13 in chick (Lin et al., 2007) and ADAM9, ADAM11 and ADAM13 in Xenopus (Alfandari et al., 2001; Cai et al., 1998). The migration of cardiac NC cells is unaffected in ADAM19-deficient mice (Komatsu et al., 2007). However, ADAM19-deficient cardiac NC cells are unable to fuse the left and right ridges of the endocardial cushion, resulting in defects in the outflow tract and formation of the ventricular septum (Komatsu et al., 2007).

Enteric NC Cells

Enteric NC-derived cells arise from two levels of the neural tube. The largest contribution of cells originates from the vagal level (adjacent to somites 1-7) (Le Douarin and Teillet, 1973; Yntema and Hammond, 1954), which overlaps with the region that gives rise to cardiac NC. A second, smaller, population of cells originates from the sacral level (caudal to somite 28) and contributes some neurons to the colon in chick and mouse (Anderson et al., 2006a; Burns and Le Douarin, 1998; Kapur, 2000; Wang et al., 2011). Defects in vagal NC migration result in variable lengths of the distal gastrointestinal tract lacking enteric neurons; a condition in humans called Hirschsprung disease (HSCR) or congenital aganglionosis. Most of the expression and functional studies of cell adhesion in enteric NC have been carried out using mice, with some data also available from Aves.

Cadherins

Enteric NC cells in mice express N-cadherin, Cadherin-11, Cadherin 9 and Cadherin-6 as well as Protocadherins α1, α4, α10 and 15 (Breau et al., 2006; Heanue and Pachnis, 2006; Vohra et al., 2006). Mice null for protocadherin 15 do not display any defects in the structure of the enteric nervous system (Vohra et al., 2006). In contrast to the early down regulation of Cadherin 6B observed in avian vagal NC cells (Nakagawa and Takeichi, 1995), murine Cadherin-6 expression is maintained in enteric NC cells as they colonise the entire length of the gut (Breau et al., 2006). Mouse and avian enteric NC express N-cadherin at low levels during migration, which is up-regulated during aggregation into ganglia (Hackett-Jones et al., 2011).
Conditional loss of N-cadherin in NC in Ht-PA-Cre mice results in a transient delay in colonization of the gut, with mutant enteric NC exhibiting perturbed directionality compared to control enteric NC and loss of the normal chain-like pattern of migration (Broders-Bondon et al., 2012). However, the delayed migration did not result in intestinal aganglionosis in the mutant mice. Double conditional mouse knockouts of N-cadherin and β1 integrin resulted in a more severe phenotype and intestinal aganglionosis (see below).

Integrins

Enteric murine and rat NC cells express several integrins, including the α4, α5, α6, αv, β1, β3 and β5 subunits (Bixby et al., 2002; Breau et al., 2009; Breau et al., 2006; Iwashita et al., 2003). The migration of enteric NC cells is impaired in β1 integrin conditional null mutant mice, resulting in absence of enteric ganglia from the distal colon (Breau et al., 2006; Pietri et al., 2003). The absence of β1 integrin also results in an abnormal organization of the enteric ganglia network (Breau et al., 2006; Pietri et al., 2003), with larger aggregations and more space between ganglia. Time-lapse imaging analysis revealed that enteric NC cells lacking β1 integrin migrated more slowly and with less persistence than control cells, most notably around the time of invasion of the caecum and hindgut (Breau et al., 2009). Fibronectin and tenascin-C are expressed at high levels in the caecum and proximal hindgut relative to the midgut. In vitro assays demonstrated that tenascin-C inhibited both adhesion and migration of enteric NC, with a stronger effect on β1 integrin null cells. In contrast, fibronectin stimulated adhesion and migration of control enteric NC, but not integrin β1-null cells. Thus β1 integrins appear to be necessary for fibronectin-mediated migration in the caecum and proximal hindgut, and to overcome the tenascin-C mediated inhibition of migration in these regions (Breau et al., 2009).

Double conditional mouse knockout of β1 integrin and N-cadherin caused a more severe intestinal aganglionosis than the single β1 integrin mutation, suggesting there is co-operation between these two adhesion molecules during enteric NC migration (Broders-Bondon et al., 2012). However, the abnormal ganglia network observed in β1 integrin mutants was partially rescued in these double mutants. Therefore, the
organization of the network of enteric ganglia requires a balance of cell-adhesion and extracellular matrix adhesion, modulated by the activity of N-cadherin and β1 integrins.

Integrin β1 is also involved in the migration of chick enteric NC along endothelial cells (Nagy et al., 2009). In chick and zebrafish, there is contact between enteric NC and blood vessels along the gut (Nagy et al., 2009). Pharmacological inhibition of endothelial cell development in cultured avian gut resulted in cessation of enteric NC migration and distal aganglionosis. However, there may be a species difference in this pattern of migration: although enteric NC are reported to be adjacent to blood vessels in chick, in mice they are not co-localised (Young et al., 2004).

The integrin β2-like gene and integrin αE1 are also expressed by murine enteric NC cells (Iwashita et al., 2003; Vohra et al., 2006), but their role in migration is not yet known.

**MMPs**

Enteric NC cells migrate in chains (Young et al., 2004). However, in order to advance, the cells must transiently weaken adhesion to other enteric NC cells. MMP2 (Anderson et al., 2006b), ADAM17 and ADAMTS1 (Iwashita et al., 2003) are all expressed by mouse enteric NC. The expression of MMPs by enteric NC-derived cells permits individual migrating cells to break away from chains (Anderson et al., 2006b). Broad spectrum inhibitors to gelatinases MMP 2 and 9 retarded enteric NC migration in explants of mouse gut, as did a specific MMP2 inhibitor (Anderson, 2010). Furthermore, the topography of the network was different in that fewer, thicker chains were present following inhibition of MMP2 and 9.

**IgCAMs**

NCAM is expressed by all enteric NC cells in mice (Heanue and Pachnis, 2006; Vohra et al., 2006) and Aves, and expression of NCAM increases following neuronal differentiation (Hackett-Jones et al., 2011). PSA modification of NCAM regulates
nerve fibre fasciculation and is involved in the clustering of individual enteric NC cells into enteric ganglia. Excessive PSA modification induced by exposure to BMP4 reduced the distance migrated by mouse NC cells in gut organ culture (Fu et al., 2006).

Like NCAM, L1CAM is also expressed by most enteric NC cells in mice (Anderson et al., 2006b) and in Aves by all enteric NC at the wavefront (Hackett-Jones et al., 2011; Nagy et al., 2012). Perturbation studies, using function blocking antibodies and recombinant protein, showed that disrupting L1 activity delays enteric NC cell migration and increases the presence of solitary NC cells close to the migratory wavefront within explants of mouse gut (Anderson et al., 2006b). The migration of enteric NC cells within the developing gut is also delayed in \textit{L1cam} deficient mice, although no change in the number of solitary cells was reported (Anderson et al., 2006b). Subsequently \textit{L1cam} has been shown to act as a modifier gene for three HSCR associated genes, \textit{Sox10}, \textit{Et3} and \textit{EdnrB}, in mice, as interactions between these genes result in a significant increase in the penetrance and/or severity of intestinal aganglionosis in mutant mice (Wallace et al., 2010; Wallace et al., 2011). L1CAM can be cleaved by ADAM10 and ADAM17, which may regulate L1-dependent cell adhesion (Maretzky et al., 2005), and while both L1CAM and ADAM17 are expressed by enteric NC in mice, it is not yet known if this occurs during enteric NC migration.

**Trunk Neural Crest**

Trunk NC cells give rise to neurons and glia in dorsal root ganglia and sympathetic ganglia, Schwann cells, boundary cap cells, cells of the adrenal medulla and melanocytes (Le Douarin and Kalcheim, 1999; Serbedzija et al., 1994; Weston, 1963). Time lapse imaging of migrating trunk NC cells in chick embryos has revealed that they maintain close, extensive and dynamic contact with other NC cells and many migrate in chains through the somites (Kasemeier-Kulesa et al., 2005).
Both N-cadherin and cadherin 6B have been shown to be involved in avian trunk NC EMT, with quite different roles. Cadherin 6B is expressed in premigratory NC and during EMT, but is absent in NC migrating away from the neural tube similarly to other axial levels (Nakagawa and Takeichi, 1995; Park and Gumbiner, 2010). Inhibition or knockdown of cadherin 6B in avian trunk NC caused a decrease in migrating NC cells, while overexpression increased the number of cells ectopically localised in the neural tube lumen (Park and Gumbiner, 2010). This effect of cadherin 6B in the de-epithelialization of the trunk NC is mediated by non-canonical BMP signaling via the BMP type II receptor, involving LIM kinase 1 which phosphorylates cofilin to regulate actin dynamics (Park and Gumbiner, 2012). While overexpression of cadherin 6B at both cranial and trunk levels promoted the ectopic localization of NC cells in the neural tube lumen, inhibition or knockdown had opposing effects on NC EMT. This may be due to differences in methodology, or may reflect inherent differences between cranial and trunk NC, similarly to the requirement for G1-S cell cycle transition, which is necessary for trunk but not cranial NC EMT (Burstyn-Cohen et al., 2004; Theveneau et al., 2007).

N-cadherin mRNA is expressed in the chick dorsal neural tube prior to and at the time of neural crest migration (Shoval et al., 2007). Overexpression many hours prior to NC emigration in ovo of full-length N-cadherin, but not dominant-negative forms, causes inhibition of trunk EMT (Shoval et al., 2007). N-cadherin can be cleaved by ADAM10, and a second cleavage step intracellularly yields a soluble cytoplasmic tail termed CTF2, which increases cytosolic β-catenin and translocates to the nucleus to regulate gene expression (Reiss et al., 2005). Blocking the action of ADAM10 in vitro inhibits chick trunk NC EMT, which can be rescued by transfection of CTF2 (Shoval et al., 2007). Electroporation of the CTF2 fragment in ovo increased EMT, and β-catenin and cyclin D1 expression (Shoval et al., 2007). These experiments suggest that during trunk NC EMT ADAM10 and N-cadherin function in cell signaling and regulation of gene transcription through the action of CTF2/β-catenin. Consistent with this, β-catenin is localized to the nucleus at the onset of migration in avian trunk NC in vitro, but during migration, it is associated with N-cadherin at
intercellular contacts (de Melker et al., 2004). However, loss of β-catenin in premigratory neural crest in Wnt1-cre mice does not inhibit NC EMT, but NC-derived melanocytes and dorsal root ganglia fail to develop, suggesting involvement of β-catenin signaling in differentiation of specific lineages rather than EMT or migration (Hari et al., 2002). Interestingly, overstimulation of β-catenin in avian trunk NC in vitro promoted translocation to the nucleus and inhibited EMT and NC migration, by decreasing integrin-mediated cell-matrix adhesion and reduced proliferation (de Melker et al., 2004), showing cross-talk between the actions of different cell adhesion and signaling pathways.

In contrast to enteric NC, N-cadherin protein does not appear to be expressed during avian trunk NC migration in vivo; expression of N-cadherin is later observed after cells coalesce to form dorsal root and sympathetic ganglia (Akitaya and Bronner-Fraser, 1992; Duband et al., 1988). However, during migration in vitro, avian trunk NC cells retain N-cadherin expression, although it is constitutively phosphorylated and is not associated with the cytoskeleton, such that N-cadherin mediated cell-cell contacts are not stable (Monier-Gavelle and Duband, 1995). The intracellular location of N-cadherin and stability of N-cadherin mediated cell-cell interactions were modified by the activity or inhibition of protein kinases and phosphatases, but the phosphorylation of N-cadherin was not affected by these protein kinases and phosphatases (Monier-Gavelle and Duband, 1995). Moreover, the localisation of N-cadherin in migrating avian NC in vitro is regulated by β1 and β3 integrin signalling (Monier-Gavelle and Duband, 1997). Decreasing integrin-mediated cell-matrix adhesion increased N-cadherin mediated intercellular adhesion in vitro (de Melker et al., 2004). It is not yet known if migrating trunk NC in vivo express N-cadherin mRNA. Recent in vivo studies showed that NC cells over-expressing full-length N-cadherin migrated more slowly, as did NC cells expressing a dominant-negative, catenin-binding domain (Kasemeier-Kulesa et al., 2006). These N-cadherin over-expressing NC cells also displayed altered morphology: increased levels of N-cadherin increased the number of filopodia, while those expressing dominant-negative N-cadherin had fewer filopodia. However, the N-cadherin over-expressing NC cells were still able to migrate ventrally to the sympathetic ganglia (Kasemeier-Kulesa et al., 2006). In contrast, N-cadherin expressing sarcoma cells failed to migrate
following transplantation into the avian trunk NC pathway \textit{in ovo}, in contrast to the parental cell line and cadherin 7 expressing cells (Dufour et al., 1999). These differing observations suggests that in trunk NC, high levels of N-cadherin expression may be regulated such that they do not completely inhibit migration. Furthermore, the similar effect of the full-length and dominant-negative N-cadherin on slowing but not abolishing migration further support the notion that increased or decreased levels of cell-cell adhesion slow neural crest migration, and shows similarities to the slowed migration of mouse enteric NC with a conditional loss of N-cadherin (see above).

Cadherin 6 is expressed in mouse NC cells prior to and during migration (Inoue et al., 1997). In Aves, cadherin 7 is expressed by the majority of trunk NC migrating in the ventral pathway to the sympathetic and dorsal root ganglia, and in the dorsolateral pathway (Nakagawa and Takeichi, 1995; 1998). It has been hypothesised that expression of cadherin 7 in a large subpopulation slows the migration of these cells, keeping them together and helping to mediate cell sorting into discrete locations and cell types (Nakagawa and Takeichi, 1995), however recent observations of cranial NC suggest cadherin 7 is expressed at higher levels in following rather than leading or pioneer cells (McLennan et al., 2012). Over-expression of cadherin 7 inhibited the emigration of some NC cells and caused ectopic localisation in the neural tube, in the same manner as over-expression of N-cadherin or cadherin 6B (Nakagawa and Takeichi, 1998; Park and Gumbiner, 2010), which is likely a general effect of increased cell-cell adhesion. Expression of cadherin 7 by sarcoma cells did not inhibit migration of these cells following transplantation to the avian trunk NC pathway (Dufour et al., 1999). The cell-cell interactions between these cadherin 7-expressing sarcoma cells \textit{in vitro} were transient and more rapidly turned over than N-cadherin mediated interactions (Dufour et al., 1999). Furthermore, the cadherin 7 and N-cadherin mediated adhesion had different effects on integrin-dependent signaling and consequent behavior of the cells on fibronectin (Dufour et al., 1999). Cadherin 11 is expressed the early migrating trunk avian NC and in the mesenchyme through which the NC migrate (Chalpe et al., 2010). The location of these cadherin 11-positive cells suggests that the most ventrally migrating trunk NC express cadherin 11, and that it is only weakly or not expressed in later migrating NC. As trunk NC aggregate into dorsal root ganglia, they only very weakly express cadherin 11, while the surrounding mesenchyme is strongly positive (Chalpe et al., 2010). Both cadherin 7 and cadherin
11 are upregulated in avian trunk NC in vitro in response to Wnt3a, at both mRNA level and protein at cell-cell adhesions (Chalpe et al., 2010), suggesting these adhesion molecules are regulated by Wnt signalling. Cadherin 11 is expressed by *Xenopus* trunk neural crest migrating in the dorsal fin (Vallin et al., 1998), and cadherin 19 is expressed in a subpopulation of trunk NC migrating in the ventral pathway in rat (Takahashi and Osumi, 2005). Overall, many questions remain to be answered on the role of type II cadherins during the migration of trunk NC.

In contrast to the expression of cadherins by NC, truncated cadherin (T-cadherin), an atypical cadherin which lacks cytoplasmic sequences, is expressed in the caudal half of each somite, which trunk NC avoid (Ranscht and Bronner-Fraser, 1991). NC avoidance of the caudal half-somite has been shown to be mediated by semaphorin-neuropilin signaling (Gammill and Roffers-Agarwal, 2010), and a role for T-cadherin in NC migration through the rostral somite has not yet been demonstrated.

Later expression of N-cadherin is required for the aggregation of NC cells in the chick sympathetic ganglia. Inhibition of N-cadherin in trunk NC by function blocking antibodies or dominant-negative constructs resulted in abnormal elongation and increased size of the sympathetic ganglia, while over-expression of N-cadherin decreased the size of the sympathetic ganglia but not length (Kasemeier-Kulesa et al., 2006). These findings demonstrate the role of N-cadherin in mediating aggregation of the ganglia and consequently ganglia size following migration.

**Integrins**

The importance of integrin signalling in NC migration has been demonstrated by manipulations of a scaffolding protein within the integrin signalling pathway, Nedd9 (Aquino et al., 2009). Loss or gain of function of Nedd9 decreased or increased respectively the extent of chick trunk NC migration. The expression and function of integrins in the migration of trunk NC has primarily been studied in cultures of avian trunk NC. These studies have shown that many integrins are expressed by trunk NC in vitro: α1β1, α3β1, α4β1, α5β1, α6β1, α7β1, α8β1, αvβ1, αvβ3, αvβ5, and a β8 (Delannet et al., 1994; Desban and Duband, 1997; Kil and Bronner-Fraser, 1996;
Testaz et al., 1999), however the pattern of expression of most integrins *in vivo* remains to be determined. The expression of integrins β1, α1, α4, α6 and α7 have, however, been examined *in vivo* in Aves (Bronner-Fraser et al., 1992a; Duband et al., 1992; Kil and Bronner-Fraser, 1996; Kil et al., 1998; Krotoski et al., 1986; Stepp et al., 1994), and some differences have been observed between expression of integrins *in vitro* and *in vivo*. Integrin α1 is expressed weakly *in vivo* (Duband et al., 1992), but strong expression is reported *in vitro* (Desban and Duband, 1997). Other integrins show dynamic changes of expression *in vivo*. Integrin α7 is observed on a subpopulation of migrating trunk NC (Kil and Bronner-Fraser, 1996), while integrin α6 is absent from many cells during migration *in vivo* (Bronner-Fraser et al., 1992a), and is expressed *in vitro* by a subpopulation of NC, in which it is mostly cytoplasmic (Bronner-Fraser et al., 1992a; Desban and Duband, 1997). During gangliogenesis, integrin α7 is expressed in the dorsal root ganglia, while α6 is expressed in the sympathetic ganglia (Kil and Bronner-Fraser, 1996). Many integrins appear to be widely distributed over the cell surface of cultured NC; however integrins αvβ1, α1 and β3 also showed localisation to focal contacts (Delannet et al., 1994; Desban and Duband, 1997; Testaz et al., 1999). The integrins expressed by trunk NC mediate migration on multiple substrates, including fibronectin, laminin and vitronectin, all of which are present along the pathways on which NC migrate *in vivo* and can support migration *in vitro* (Duband and Thiery, 1987; Krotoski and Bronner-Fraser, 1990; Newgreen and Thiery, 1980; Rovasio et al., 1983).

The function of different integrins in mediating migration of avian trunk NC has been mostly addressed by culturing trunk NC on different substrates and using function-blocking antibodies or antisense oligonucleotides to perturb particular integrins. These experiments have revealed that different integrins regulate specific aspects of cell behaviour: some integrins play a role in mediating migration of the NC on particular substrates, while others are involved in adhesion or spreading of the NC and some do not appear to mediate any of these functions. The integrins implicated in migration include: integrin αvβ3 on fibronectin (Testaz et al., 1999), vitronectin (Delannet et al., 1994) and laminin-1 (Desban and Duband, 1997), αvβ5 on vitronectin (Delannet et al., 1994), and the β1 integrin subunit can pair with α4, α8 or α3 to mediate migration on fibronectin (Lallier and Bronner-Fraser, 1993; Testaz et
Knocking out α1 or β3 integrins in vivo in mice does not cause an abnormal neural crest migration phenotype (Gardner et al., 1996; Hodivala-Dilke et al., 1999), which may reflect functional redundancy (see below). The roles of α4, α5 and β1 subunits have been complicated by the early embryonic lethality of these knockouts (Fassler and Meyer, 1995; Goh et al., 1997; Yang et al., 1995). Some cells lacking β1 integrin can migrate to NC derivatives in chimaeric β1 null mice or following antisense blocking of β1 in chick (Fassler and Meyer, 1995; Tucker, 2004). However it is possible that these β1 integrin null cells were able to migrate due to the presence of wild-type β1 integrin expressing cells. Ht-PA-Cre conditional β1 null mice show normal trunk NC migration, but these cells still retain a low level of β1 integrin expression during NC migration (Pietri et al., 2004). Wnt1-Cre conditional β1 null mice also showed migration of trunk NC; however the extent of migration and number of migratory cells was not examined (Turlo et al., 2012). Integrins α4 and α5 are expressed by mouse trunk NC (Goh et al., 1997; Kil et al., 1998), and in α4 null mice, trunk NC migration and early gangliogenesis appear normal (Yang et al., 1995), however α5 null mice die during trunk NC migration (Goh et al., 1997). To study the importance of α4 or α5 integrins, NC null for either integrin were examined in culture, and also grafted into chick NC pathways. Integrin α5 null NC cells migrated poorly on fibronectin, but well on vitronectin, whereas α4 null cells showed normal migration on fibronectin (Haack and Hynes, 2001). This is in contrast to in vitro studies, which show an inhibition of migration using function blocking antibodies to α4 in chick and mouse (Kil et al., 1998; Testaz and Duband, 2001), and no effect on migration using function blocking antibodies to α5 (Testaz et al., 1999). When grafted into chick embryos, many α4-null glial progenitor cells underwent apoptosis during migration, especially those migrating dorsally, however some were able to migrate and contribute to the peripheral nervous system (Haack and Hynes, 2001). Blocking α4β1 integrin in cultured chick NC also results in a large increase in apoptosis (Testaz and Duband, 2001). Integrin α5 null cells migrate well in chick NC pathways, although the cells did not survive in the glial lineage (Haack and Hynes,
Although there are some discrepancies in the behavior of null cells compared to function-blocking antibody studies, overall the data suggest that a lack of integrin α5 alone does not impair trunk NC migration and integrin α4 may play a role in mediating NC survival.

In summary, trunk NC express a wide variety of integrins during migration, and multiple substrates are present along NC pathways \textit{in vivo}. There is likely to be significant functional redundancy as the loss of single integrin subunits does not reduce migration \textit{in vivo}, and blocking of multiple integrins is required to completely prevent NC migration on fibronectin: either blocking of both β1 and αvβ3, or α3, α4 and αv combined (Testaz et al., 1999). Combined knockdown studies of multiple integrins \textit{in vivo}, particularly β1 and αv, may be necessary to gain a greater understanding of the role of integrins during trunk NC migration. Further complicating the analysis of integrin function is cross-talk that occurs between different integrins, by which integrins can regulate the functioning and expression of other integrins (Retta et al., 2001; Testaz and Duband, 2001). In addition, \textit{in vitro} work using avian NC demonstrated regulation of integrin activity in response to different extracellular environments (see Cranial Neural Crest section above), with responses differing between cranial and trunk NC (Strachan and Condic, 2003; 2004; 2008). Lastly, expression of particular integrin subunits, α4 and α5, may be required for cell survival rather than, or in addition to, mediating migration, highlighting the importance of integrin-mediated signaling during NC development.

\textit{MMPs}

ADAM10 is expressed in the dorsal neural tube in the avian trunk, and in the ectoderm, dermatome and myotome, and in trunk NC in culture (Hall and Erickson, 2003). ADAM10 expression in the dorsal neural tube cleaves N-cadherin and plays a role in regulating NC EMT as described above (Shoval et al., 2007); however roles for ADAM family members in regulating trunk NC migration have not been demonstrated.
Multiple MMPs are expressed by migrating trunk NC, including MMP8 in mouse NC (Giambernardi et al., 2001), MMP2 weakly in avian NC (Duong and Erickson, 2004) or *Xenopus* mesenchyme (Tomlinson et al., 2009), MMP14 in migrating *Xenopus* NC (Harrison et al., 2004), and MMP9 in avian premigratory and migratory NC (Monsonego-Ornan et al., 2012). The function of these MMPs shows some differences between species; MMPs may be required for NC EMT in chick but not in *Xenopus*. Chemical inhibition of MMP activity or knocking down MMP2 in chick by morpholino prior to NC EMT reduced EMT and migration *in vitro* and *in ovo*, while knockdown in NC cells just emerging from the neural tube did not inhibit their migration (Duong and Erickson, 2004). Similarly, inhibition of MMP9 or use of MMP9 morpholinos reduced the number of migrating trunk NC, while overexpression or presence of MMP9 conditioned media increased the number of migratory cells and promoted premature EMT of trunk NC in chick in culture and *in ovo* (Monsonego-Ornan et al., 2012). This effect may be due to degradation of laminin in the basement membrane (Monsonego-Ornan et al., 2012). In contrast, *in vivo* knockdown or inhibition of either MMP2 or MMP14 or both in *Xenopus* perturbed the migration of melanophores, but no effect on EMT was observed, nor was the migration of other NC cells affected (Tomlinson et al., 2009).

IgCAMs

NCAM is expressed in the neural tube, and remains in avian trunk NC cells as they undergo EMT and begin to migrate, but is gradually down-regulated during migration (Akitaya and Bronner-Fraser, 1992), before being later upregulated in the ganglia (Duband et al., 1985; Lallier and Bronner-Fraser, 1988; Thiery et al., 1982). Whether NCAM expression during the early phase of trunk NC migration is important is not yet understood. In contrast to the downregulation of NCAM during NC migration, Melanoma Cell Adhesion Molecule (MCAM, also known as gicerin, HEMCAM and CD146) is expressed in migrating avian trunk NC (Alais et al., 2001). MCAM can regulate adhesion by controlling expression of integrins (Alais et al., 2001), and is upregulated during melanoma metastasis (Lehmann et al., 1987; Luca et al., 1993; Xie et al., 1997), but its function in NC migration is unknown.
Conclusions

In summary, the process of cell migration requires interactions between the migrating cells and the surrounding environment, consisting of neighboring cells and extracellular matrix molecules. An optimal level of cell adhesion is required for migration, as too little adhesion does not generate sufficient traction for motility, while too much adhesion will result in the cells remaining stationary. The families of cell adhesion molecules involved in NC migration, cadherins, integrins and the immunoglobulin superfamily, regulate cell-cell and/or cell-matrix adhesion, and are also important components of various cell signaling pathways that affect many aspects of cell behavior including proliferation, cytoskeleton dynamics and gene transcription. The function of the adhesion molecules can be regulated both intracellularly and extracellularly. Various intracellular signaling pathways can regulate the activity, membrane localization and trafficking, and hence cell adhesion function of the adhesion molecules. Furthermore, activity of one family of adhesion molecules can influence the function of other adhesion molecules, such as integrins and cadherins. Extracellularly, cleavage by matrix metalloproteases can affect the function of cell adhesion molecules, not only by reducing the level of adhesion, but also by releasing the adhesion molecule for intracellular signaling functions, such as the cleavage of N-cadherin by ADAM10 during trunk NC EMT. In addition, NC cells can respond to differences in the extracellular environment to which they are exposed by altering their activity, expression and/or localization of adhesion molecules, such as integrins. Consequently, the regulation and function of cell adhesion molecules during NC migration is complex and multifaceted.

Understanding how NC cells use cell adhesion molecules to migrate has important implications for understanding the etiology of some neurocristopathies, and the mechanisms neural crest-derived cancerous cells may use to migrate during metastasis. Over the past thirty years, the expression pattern and function of numerous cell adhesion molecules during neural crest migration has been elucidated. There are, however, many gaps in our knowledge and understanding of the role of these cell adhesion molecules during the process of migration, particularly in vivo.
Along the embryonic axis there are differences in the extracellular substrates and pathways available to NC, differences in derivatives formed and even differences in mechanisms of EMT. Therefore, the cell adhesion molecules and molecular mechanisms used by the NC to undergo EMT and migrate are likely to reflect this. Some cell adhesion molecules appear to have similar functions at all axial levels, such as MMP9, which, when inhibited in chick embryos causes a reduction in NC migration at both cranial and trunk levels. Other cell adhesion molecules appear to play a role at only one axial level, for example L1CAM, expressed by the enteric NC in mice and chick. Loss or inhibition of L1CAM slows the colonization of the gut by enteric NC in mice. In addition, NC from a single axial level migrate to multiple target sites and give rise to different types of derivatives. Several cell adhesion molecules, such as some integrins and cadherins, appear to be restricted to subpopulations of NC within an axial level, but the function of these differences is not yet clear. Recent data suggest that the level of expression of some integrins and cadherins differs depending on whether the cell is a leading or following cell, that this expression is plastic and likely reflects different mechanisms of migration between leading and following cells (McLennan et al., 2012). There are both differences and similarities in the cell adhesion molecules employed by the NC in different species. Some of these variations may reflect different mechanisms by which NC cells use to migrate in different species. Other variations may reflect different members of a gene family playing a similar role in multiple species.

Many of the studies carried out on NC cells have been conducted in vitro, particularly with the integrins. This was done out of necessity, and these studies have been very informative. However, there are limitations in the application of these results to NC migration in vivo. Another complication to understanding the role of cell adhesion molecules in vivo is functional redundancy or compensation between members of a family, and in the case of integrins, cross talk between integrins expressed by a single cell. These factors can make it very difficult to tease out the role of particular molecules. Applying some of the newer technologies to these questions may provide important insights. For example, the use of knockdown technologies, fluorescent tagging of particular cell adhesion molecules or their intracellular partners, in vivo imaging and other technologies have great potential for advances in this field, to give
us a much greater understanding of how these cell adhesion molecules function during NC cell migration.

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References


peripheral nervous system regulate the generation of neural diversity. Neuron 35, 643-56.


Highlights

- Migrating neural crest cells express a variety of cell adhesion molecules
- Expression of adhesion molecules can vary between axial levels and species
- Cleaving of cell adhesion molecules by matrix metalloproteases can affect migration
- Function of adhesion molecules can be similar or different between axial levels
Table 1: Expression of the major classes cell adhesion molecules in NC by axial level and species. Underlining or bolding indicates a function for the molecule during NC migration has been demonstrated *in vitro* or *in vivo* respectively.

<table>
<thead>
<tr>
<th>Axial level of NC</th>
<th>Rodent</th>
<th>Avian</th>
<th>Xenopus</th>
<th>Rodent</th>
<th>Avian</th>
<th>Xenopus</th>
<th>Rodent</th>
<th>Avian</th>
<th>Xenopus</th>
<th>Rodent</th>
<th>Avian</th>
<th>Xenopus</th>
<th>IgCAMs</th>
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<tr>
<td>Cranial</td>
<td>Cadherin-6 $^1$</td>
<td>Cadherin-11 $^2$</td>
<td>Cadherin-19 (rat) $^3$</td>
<td>Cadherin-6B</td>
<td>Cadherin-11</td>
<td>PCNS $^9$</td>
<td>N-cadherin $^7$</td>
<td>N-cadherin $^7$</td>
<td>N-cadherin (weak)</td>
<td>ADAM10</td>
<td>ADAM13</td>
<td>ADAM9</td>
<td></td>
</tr>
<tr>
<td>Vagal-cardiac</td>
<td>Cadherin 6</td>
<td>Cadherin 6B (EMT)</td>
<td>Cadherin 11</td>
<td>Cadherin 6</td>
<td>Cadherin 11</td>
<td>MMP2 $^{25}$</td>
<td>MMP9 $^{26}$</td>
<td>ADAM19</td>
<td>MMP2 $^{30}$</td>
<td>MMP14 $^{31}$</td>
<td></td>
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<tr>
<td>Vagal-enteric</td>
<td>N-cadherin $^{39,40,41,42}$</td>
<td>Cadherin 6</td>
<td>N-cadherin $^{43}$</td>
<td>N-cadherin $^{44}$</td>
<td>N-cadherin $^{45}$</td>
<td>N-cadherin $^{46}$</td>
<td>ADAM1</td>
<td>ADAM11</td>
<td>NCAM $^{49}$</td>
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*Note: The table entries are not listed in the image.*
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<th>Cadherin 6B (EMT)</th>
<th>MMP2 (in NC or pathway)</th>
<th>MMP8</th>
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<th>MMP2 (in pathway)</th>
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<th>NCAM (down-reg)</th>
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References: 39, 41, 13, 35, 30, 42, 47, 43, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 64.
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