Developmental Biology 444 (2018) S144–S155

Contents lists available at ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/locate/developmentalbiology

AKT signaling displays multifaceted functions in neural crest development

Méghane Sittewelle[a,b], Anne H. Monsoro-Burq[a,b,c,*]

[a] Univ. Paris Sud, Université Paris Saclay, CNRS UMR 3347, INSERM U1021, Centre Universitaire, 15, rue Georges Clémenceau, F-91405 Orsay, France
[b] Institut Curie Research Division, PSL Research University, CNRS UMR 3347, INSERM U1021, F-91405 Orsay, France
[c] Institut Universitaire de France, F-75005 Paris, France

ARTICLE INFO

Keywords:
Neural crest
Development
Ectoderm
EMT
Migration
Differentiation
AKT
PI3K
PTEN
Xenopus laevis embryo
Mouse embryo
Zebrafish embryo
PDGF
FGF
EGF

ABSTRACT

AKT signaling is an essential intracellular pathway controlling cell homeostasis, cell proliferation and survival, as well as cell migration and differentiation in adults. Alterations impacting the AKT pathway are involved in many pathological conditions in human disease. Similarly, during development, multiple transmembrane molecules, such as FGF receptors, PDGF receptors or integrins, activate AKT to control embryonic cell proliferation, migration, differentiation, and also cell fate decisions. While many studies in mouse embryos have clearly implicated AKT signaling in the differentiation of several neural crest derivatives, information on AKT functions during the earliest steps of neural crest development had remained relatively scarce until recently. However, recent studies on known and novel regulators of AKT signaling demonstrate that this pathway plays critical roles throughout the development of neural crest progenitors. Non-mammalian models such as fish and frog embryos have been instrumental to our understanding of AKT functions in neural crest development, both in neural crest progenitors and in the neighboring tissues. This review combines current knowledge acquired from all these different vertebrate animal models to describe the various roles of AKT signaling related to neural crest development in vivo. We first describe the importance of AKT signaling in patterning the tissues involved in neural crest induction, namely the dorsal mesoderm and the ectoderm. We then focus on AKT signaling functions in neural crest migration and differentiation.

1. Introduction

Neural crest cells are multipotent progenitors in the vertebrate embryo that give rise to a vast array of different cell types including pigment cells, craniofacial skeleton and mesenchyme, peripheral neurons and glia, cardiac and adrenal medulla derivatives (Bronner and LeDouarin, 2012; Kirby and Hutson, 2010). The presumptive neural crest (NC) is induced during gastrulation and neurulation, within a broad frontier area located across the neural plate and the nonneural ectoderm, named the neural (plate) border (NB) (see review by Pla and Monsoro-Burq, 2018 this issue). This domain is a mixed territory, which also contributes to the dorsal neural tube, the nonneural ectoderm and, anteriorly, to cranial placode progenitors (Steventon et al., 2009; Streit, 2002; reviewed in Pegoraro and Monsoro-Burq, 2013). The NB is induced by a combination of signals secreted from the adjacent tissues, namely the neural and non-neural dorsal ectoderm and the underlying paraxial and intermediate mesoderm. FGF signals, diffusing from the mesoderm, and Wnt ligands from both non-neural ectoderm and mesoderm, cooperate with low-level BMP signaling to activate the expression of the neural border specifiers *pax3/7, zic1/2, tfap2a, hes4*, or *msx1/2*, which are transcription factors required to establish the NB territory and are necessary for NC development (Basch and Bronner-Fraser, 2006; de Crozé et al., 2011; Mizuseki et al., 1998; Monsoro-Burq et al., 2005; Nichane et al., 2008a; Sasai, 2005; Tribulo, 2003). In turn, these factors synergize with WNT signals to activate the NC developmental program (Monsoro-Burq et al., 2005; Sasai, 2005; Simões-Costa et al., 2015). Further NC development follows successive steps: induction, epithelial-to-mesenchymal transition (EMT), migration and differentiation. This complex process is controlled by a network of transcription factors and soluble molecules: the NC gene regulatory network (NC-GRN), which has been validated in vivo, in multiple animal models including frog, chick and fish for the early stages of the network (Garnett et al., 2012; Milet and Monsoro-Burq, 2012; Sauka-Spengler and Bronner-Fraser, 2008). Multiple transcription factors are expressed and cooperate at each step of the network; for example, *snai2, foxd3, sox8/9/10, twist1, ets1* are expressed in premigratory NC and are essential for NC specification and EMT. As our knowledge of the NC-GRN deepens, the main phases of NC formation can be subdivided into more precise sub-steps. To illustrate, during premigratory NC

* Corresponding author at: Univ. Paris Sud, Université Paris Saclay, CNRS UMR 3347, INSERM U1021, Centre Universitaire, 15, rue Georges Clémenceau, F-91405 Orsay, France.
E-mail address: anne-helene.monsoro-burq@curie.fr (A.H. Monsoro-Burq).

https://doi.org/10.1016/j.ydbio.2018.05.023
Received 21 February 2018; Received in revised form 24 May 2018; Accepted 29 May 2018
Available online 31 May 2018

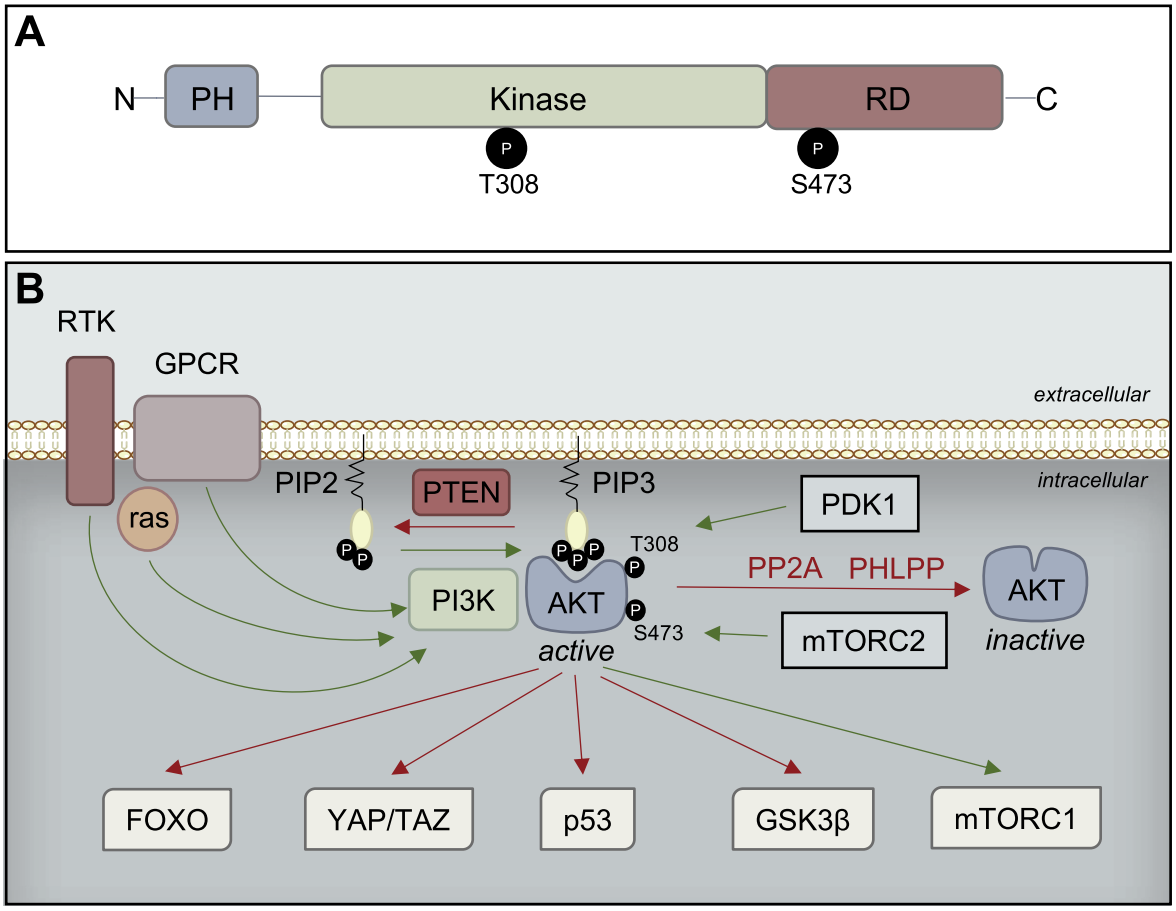

Fig. 1. Overview of the AKT signaling pathway. (A) Schematic structure of the AKT protein. AKT contain three main protein domains: the N-terminal pleckstrin homology (PH) domain, the catalytic domain (kinase) and the hydrophobic C-terminal regulatory domain (RD). The two major phosphorylation sites T308 and S473, needed for the full activation of AKT are indicated. (B) Outline of AKT regulation and major downstream targets during development, as discussed in the text. The activation of PI3K leads to PIP3 production and accumulation at the plasma membrane. PTEN catalyzes the reverse reaction thus regulates AKT activation negatively. AKT is recruited at the plasma membrane via its PH domain and undergoes full activation through two sequential phosphorylations: T308 phosphorylation (by PDK1) and S473 phosphorylation (by mTORC2). PP2A and PHLPP are phosphatases able to directly dephosphorylate these two phosphorylation sites reverting AKT to an inactive conformation. The main potential downstream effectors of AKT signaling are indicated. Green arrows indicate a positive regulation; red arrows indicate a negative regulation.

specification, two phases can be distinguished: first, the early premigratory NC cells (NCC), marked by expression of the transcription factors *snai2, sox8* and *foxd3*, are induced and the immature NC population is maintained and amplified by action of BMP, Wnt and Notch signaling (Faure et al., 2002; Garcia-Castro et al., 2002; Nichane et al., 2008a, 2008b); then, at neural fold stage, mature premigratory NCC express later NC specifiers, such as *sox10* and *twist*, and activate numerous cell modifications that are essential for NCC EMT and migration.

Until recently, very little evidence had implicated AKT signaling in NC early development. However, several new studies in fish, frog and mouse embryos have shown an essential role for AKT at each step of NC formation, from induction to differentiation. The serine/threonine kinase AKT, also called PKB for protein kinase B, is a major node in cell signaling for cell homeostasis. AKT protein comprises 4 main domains: a N-terminal pleckstrin homology (PH) domain, which recognizes and binds phosphatidylinositol-3,4,5-trisphosphate (PIP3) and allows AKT recruitment at the plasma membrane; a linker domain, a kinase domain and a C-terminal regulatory domain (Fig. 1A; Manning and Toker, 2017; Ruan and Kazlauskas, 2011). AKT acts downstream of signaling events recruiting PI3-kinase (PI3K) at the plasma membrane, such as the activation of receptor tyrosine kinases (e.g. PDGFRs, FGFRs, EGFRs, insulin receptors), of integrins, of B and T cell receptors, of cytokine receptors, or of G-protein-coupled receptors (Hers et al., 2011; Manning and Cantley, 2007; Manning and Toker, 2017). PI3K phosphorylates phosphatidyl-inositol-4,5-bisphosphate (PIP2) into PIP3, while the phosphatase PTEN catalyzes the reverse reaction (Divecha and Irvine, 1995; Maehama and Dixon, 1998). The accumulation of PIP3 at the plasma membrane simultaneously recruits AKT and PDK1 (3-phosphoinositide-dependent protein kinase 1), via their respective PH-domains proteins (Fig. 1B). AKT is then activated by two sequential phosphorylation events. First, PDK1 phosphorylates AKT on Thr-308, which stabilizes the activation loop located in the kinase domain. Secondly, the complex mTORC2 phosphorylates AKT on Ser-473, located in the C-terminal regulatory domain (Alessi et al., 1997, 1996; Sarbassov et al., 2005; Stokoe et al., 1997). These two phosphorylation events are essential to attain maximal AKT activation. Moreover, many other post-translational modifications, including additional phosphorylations on Ser/Thr/Tyr residues, acetylation/ubiquitylation/methylation on lysine residues, hydroxylation, glycosylation, and SUMOylation participate in the fine tuning of AKT stability, activity, sub-cellular localization and partner selection (Risso et al., 2015). In turn, AKT controls multiple downstream effector pathways to monitor various major cellular functions. Among over a hundred different substrates reported by in vitro or in vivo experiments, active AKT controls the activity of FoxO proteins, regulators of cell survival, proliferation, growth and metabolism; of GSK3 which has major roles in survival, proliferation, metabolism and neural development; of mTORC1 known to act in protein synthesis and growth signaling; of p53 controlling cell cycle but also cell survival migration and metabolism; and of YAP broadly involved in proliferation, survival, growth and differentiation (Ma et al., 2007; Manning and Cantley, 2007; Moroishi

et al., 2015; Piccolo et al., 2014; Roger et al., 2006; Tokino and Nakamura, 2000; Fig. 1B). Several of these targets have been studied in NC development, albeit not specifically as effectors of AKT signaling. GSK3 is a negative regulator of the Wnt-βcatenin signaling pathway, essential for NC development (see below). In frog embryos, FoxO4 depletion leads to craniofacial and pigmentation defects (Schuff et al., 2010). Defective p53 expression causes craniofacial defect in fish, mouse and chick (Rinon et al., 2011; Xia et al., 2013). Finally, YAP is important for mouse craniofacial development (Ma et al., 2007; Manning and Cantley, 2007; Moroishi et al., 2015; Piccolo et al., 2014; Roger et al., 2006; Tokino and Nakamura, 2000; Wang et al., 2016) as well as for NC stemness in human NCC in vitro (Hindley et al., 2016).

This review describes the novel roles of AKT signaling, revealed by recent studies in frog, fish and mouse models, during the successive developmental steps that lead to neural crest formation in vivo, from gastrulation to organogenesis. We highlight how known and novel inputs control AKT activation during essential patterning events acting upstream of NC formation, during cleavage and gastrulation stages; then how AKT signaling controls premigratory NC induction and maturation during neurulation, NC EMT and migration at the end of neurulation, and NC differentiation into different cell types during organogenesis.

2. AKT signaling is essential during early dorsal patterning, neural crest induction and maturation

2.1. AKT signaling in dorsal mesoderm induction and in gastrulation

During embryogenesis, AKT signaling is involved in a sequence of early patterning events occurring upstream of NC formation, namely mesoderm induction and dorsal ectoderm patterning, which, as mentioned above, are prerequisite steps upstream of NB and NC formation. The amenability of cleavage-stage and gastrula-stage frog embryos to study early patterning events, notably germ layer specification, has been instrumental in those studies. Various approaches conducted in vivo have thus been used to modulate PI3K-AKT signaling, either using activated or dominant-negative forms of these kinases, or, more precisely, using time-controlled inactivation with a pharmacological compound inhibiting PI3K activity (LY294002). Whole embryo treatment with LY294002, from late cleavage stage to the end of gastrulation, blocked the movements of gastrulation and prevented the complete internalization of mesoderm and endoderm. The resulting embryos lack head and axial mesoderm: the differentiation markers for paraxial muscle (12/101) and notochord (MZ15) were strongly decreased compared to control sibling embryos at tailbud stage. Early trunk mesoderm markers (*brachyury (xbra)*, *apod*, *not1*) failed to be activated at early gastrula stage, while the markers of early endoderm (*mixer*, *sox17a*) and organizer (*siamois*, *gsc*, *chd*) were expressed similarly in treated embryos and controls (Carballada et al., 2001).

During mesoderm induction, the PI3K-AKT pathway is required in response to FGF signaling. A classical model for studying mesoderm induction is to use the pluripotent animal cap cells dissected out of frog blastulae and grown in vitro with soluble mesoderm inducers. When treated either with activin or FGF, these explants differentiate into dorsal mesoderm. They first activate *xbra* expression, then muscle differentiation markers and undergo convergent and extension movements, resulting in explants with an elongated shape (Amaya et al., 1993; O'Reilly et al., 1995). However, if a similar experiment is done in presence of both FGF and LY294002, the early induction of *brachyury* and the elongation movements are blocked (Carballada et al., 2001). Moreover, because FGF-mediated mesoderm induction is known to require the Mitogen Activated Protein Kinase (MAPK) pathway, the relationships between the MAPK and PI3K intracellular pathways were studied in similar animal cap assays. An efficient activation of ERK is obtained when animal caps are treated with FGF, even in the presence of LY294002, showing that PI3K activity does not affect MAPK signaling in this context (Carballada et al., 2001; Peng et al., 2004; Fig. 2). However, in contrast to FGF stimulation, PI3K-AKT constitutive activation (ca) alone results in low-level *brachyury* induction, even when high doses of caPI3K are used. Simultaneous activation of caPI3K and caERK improves the efficiency of mesoderm induction, with formation of ventral mesoderm (mesothelium), although dorsal mesoderm, identified by high levels of *brachyury* expression, failed to be activated in this assay. Together, these results show that both PI3K-AKT and MAPK pathways are required to mediate mesoderm induction by FGF. The two pathways act in parallel since blocking PI3K-AKT does not alter MAPK signaling. However, as the co-activation of these two intracellular activities fails to activate high *brachyury* expression, this suggests that additional signals are needed for the full activity of FGF signaling during dorsal mesoderm induction.

As described above, when PI3K signaling is inhibited in during late blastula stages, the trunk dorsal mesoderm (muscle and notochord) fails to be induced, and the dorsal embryonic axis does not form (Carballada et al., 2001; Peng et al., 2004). In contrast, when a constitutively active (ca) form of PI3K or of caAKT is expressed ectopically, by injections at the animal pole of 2-cell stage embryos, a secondary dorsal axis forms, resulting in twin embryos (Peng et al., 2004). Classical experiments on dorsal axis patterning have shown that the ectopic activation of Wnt-β-catenin signaling causes a similar double dorsal axis phenotype which can be suppressed by overexpression of GSK3β (McMahon and Moon, 1989; Larabell, 1997; Glinka et al., 1998). Similarly, the double axis phenotype caused by ectopic caAKT activity is rescued by GSK3β gain-of-function (Peng et al., 2004). Given that AKT can inactivate GSK3β activity by phosphorylation, it has been proposed that AKT may participate in dorsal axis formation by inhibiting GSK3β and thus potentiating β-catenin activity (Peng et al., 2004; Yang et al., 2008; Fig. 2).

Finally, of the putative AKT regulators mentioned above, EGFR has also been suggested to play a role in the early embryo. In contrast to FGF receptor blockade, the inhibition of EGFR/Erb1-4 signaling does not prevent mesoderm induction. However, similarly to FGF signaling, depletion of ErbB4 also blocks gastrulation movements by affecting cell-cell adhesion and cell motility (Nie and Chang, 2007a). Upon depletion of ErbB4, constitutive activation of either PI3K or AKT can rescue convergence-extension movements both in whole embryos and in mesoderm explants, as well as cell-cell adhesion and motility characteristics (Nie and Chang, 2007b). Therefore, PI3K-AKT signals are essential for the induction of the dorsal mesoderm, downstream of FGF signaling, in late blastulae as well as the movements of gastrulation driven by dorsal mesoderm cells, downstream of FGF and EGF receptors.

2.2. AKT signaling in dorsal ectoderm patterning

During gastrulation, the dorsal mesoderm is progressively positioned under the dorsal ectoderm. Both neural induction and the induction of the neural border are initiated simultaneously, as gastrulation starts with the ingression of dorsal and paraxial/intermediate mesoderm respectively (Harland, 2000). Neural plate markers (*sox2*) and NB markers (*pax3/7*) are readily detected by this stage (Basch and Bronner-Fraser, 2006; de Crozé et al., 2011; Monsoro-Burq et al., 2005; Plouhinec et al., 2017). When PI3K/AKT signaling is blocked during gastrulation (i.e. at a later developmental stage than in the experiments described above), partially defective dorsal mesoderm patterning is observed: the notochord is reduced and muscle formation is diminished (Carballada et al., 2001). This phenotype could be sufficient to cause subsequent defective neural and neural border induction. In order to try and separate the roles of AKT signaling in neural induction from its functions in mesoderm induction and morphogenesis, PI3K-AKT signals have been activated in animal cap explants in vitro. When, caPI3K or caAKT are injected in animal caps,

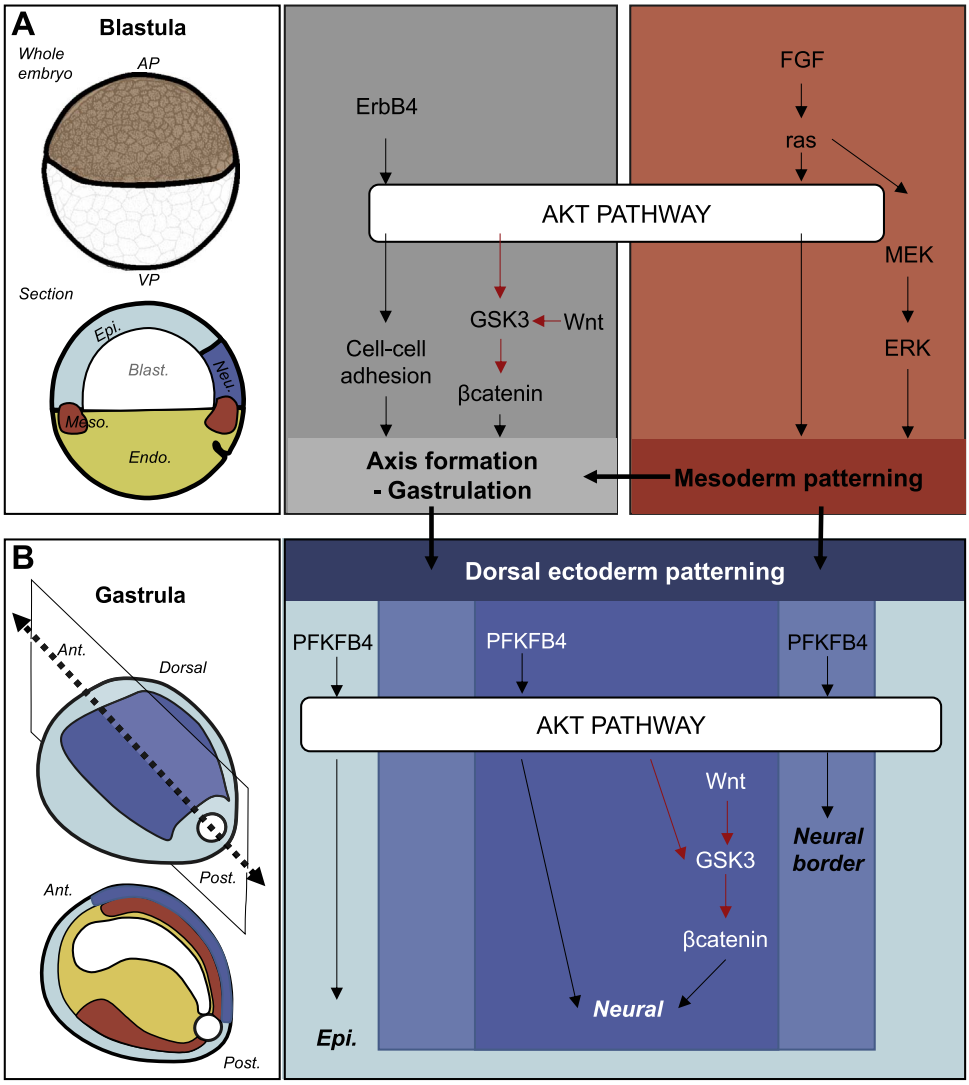

Fig. 2. Regulations linked to AKT signaling during early embryo dorsal patterning. (A) On the left, representation of a whole blastula in external view and in sagittal section. The three germ layers and the blastocoel (Blast.) are indicated: the ectoderm in blue, the mesoderm (Meso.) in red, and the endoderm (Endo.) in yellow. The ectoderm is further subdivided into two parts: the neural plate (Neu.) in dark blue and the nonneural ectoderm (Epi.) in light blue. On the right, schematic representation of the regulations involving the AKT pathway during mesoderm patterning, dorsal axis formation and gastrulation. (B) On the left, representation of a late gastrula, in external view and in sagittal section. The same color code for different germ layers is used. On the right, schematic representation of regulations linked to AKT signaling regulating dorsal ectoderm patterning. See text for details. Animal pole (AP); Vegetal pole (VP). Red arrows indicate negative regulations.

this is sufficient to trigger the expression of the pan-neural marker *ncam* and the regional anterior and posterior neural markers *otx2* (midbrain) and *hoxb9* (spinal cord) (Peng et al., 2004; Yang et al., 2008). The induction of neural markers occurs in the absence of *brachyury* induction. This indicates that PI3K-AKT signaling activation triggers neural induction and regionalization independently of mesoderm induction.

Besides growth factor receptors and known AKT regulators, novel molecules controlling AKT phosphorylation have been recently identified. For example, our laboratory has found a crucial role for an enzyme that regulates the rate of glycolysis, 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase (PFKFB). This enzyme controls AKT signaling during ectoderm development. In gastrula-stage frog embryos, PFKFB4 is expressed broadly in the dorsal ectoderm that will form the neural plate, the neural border, and part of the nonneural ectoderm (Pegoraro et al., 2015; Pegoraro and Monsoro-Burq, 2013). The depletion of PFKFB4 in vivo, using antisense morpholino oligonucleotides, blocks ectoderm patterning during gastrulation by preventing the activation of all dorsal ectoderm markers, such as *sox2* (neural plate), *snai2* (NC), *six1* (pan-placode ectoderm) and *epidermal keratin* (non-neural ectoderm) (Fig. 2). Instead, the ectodermal cells retain an immature progenitor status, with high expression of markers such as *sox3* (immature neural ectoderm), *pax3, hes4* (early NB) and *foxi1e* (immature non-neural ectoderm) (de Crozé et al., 2011; Mir et al., 2007; Nichane et al., 2008a; Wang et al., 2006). In this context, neurulation movements are blocked. However, in contrast to the severe phenotype of PFKFB4-depleted embryos, glycolysis blockade does not perturb ectoderm development and neurulation (Pegoraro et al., 2015). This is consistent with the fact that, in early frog embryos, glycolysis is not required for development because the cell energy metabolism is fueled by yolk-derived amino acids entering the citric acid cycle (Vastag et al., 2011). The discrepancy between the phenotype of PFKFB4-depleted embryos and that of glycolysis-blocked embryos lead to the hypothesis that PFKFB enzymes also play a non-conventional, glycolysis-independent, function. A candidate signaling pathway approach revealed that PFKFB4 is required to phosphorylate AKT in the dorsal part of the embryo (Pegoraro et al., 2015). Further experiments link PFKFB4 and AKT signaling. Both PFKFB4 depletion and time-controlled PI3K-AKT pharmacological inhibition display similar phenotypes on the patterning of the ectoderm during gastrulation, using the

markers described above. In this case, LY294002 was applied from the start of gastrulation to mid-neural plate stage, to avoid perturbation of mesoderm induction and to limit the effects on gastrulation movements. Of note, PFKFB4 depletion does not significantly alter mesoderm induction and positioning during gastrulation, in agreement with restriction of its role to the ectoderm layer. Finally, the constitutive activation of AKT rescues the PFKFB4 depletion phenotype, demonstrating that AKT signaling specifically mediates PFKFB4 functions in early dorsal ectoderm global development (Pegoraro et al., 2015). Together, these studies demonstrate the important role of PI3K-AKT signals to establish the neural and neural border territories in the dorsal ectoderm, upstream of neural crest induction. They also highlight the essential tissue-specific activation of the various regulators of AKT signaling which are required to finely tune the levels of this broad-spectrum pathway in specific target cells.

2.3. AKT signaling in early neural crest patterning

Based on work in multiple vertebrate species, such as amphibians, fish, lamprey and chick, the premigratory neural crest is established in at least two main steps after neural border formation (Aybar and Mayor, 2002; Germanguz et al., 2007; Nikitina and Bronner-Fraser, 2009; Pegoraro and Monsoro-Burq, 2013). Firstly, the earliest NC markers, such as *snai2, foxd3 and sox8,* are induced at the open neural plate stage. Then, during the progressive elevation of the neural folds, a second set of characteristic NC molecules, such as *twist1* and *sox10,* are activated, preparing the cellular modifications of epithelial-mesenchymal transition (EMT), such as the switch of cadherin expression and activation of metalloproteases. Finally, EMT occurs around the time of neural tube closure. Three recent studies, conducted in fish and in frog embryos, have implicated AKT in premigratory neural crest development, either during its induction at the edges of the neural plate, or during the maturation of the premigratory neural crest at neural fold stage or during EMT (Neuner et al., 2009; Figueiredo et al., 2017; Ciarlo et al., 2017). Importantly, these studies have been conducted after the establishment of the neural plate, or in experimental conditions that do not cause significant defects in mesoderm induction, in gastrulation movements, in neural induction or in NB initiation.

The first series of indirect evidence involves the ADAM19 disintegrin metalloprotease, which is expressed in the dorsal mesoderm, the neurectoderm and neural crest cells of the developing frog embryo (Neuner et al., 2009). In many cellular and in vivo models, ADAM19 is involved in processing pro-heparin-binding EGF (HB-EGF) and neuregulin, upstream of the EGFR receptor tyrosine kinase (Horiuchi et al., 2005; Kurohara et al., 2004; Shirakabe et al., 2000; Taylor et al., 2014). In whole frog embryos, ADAM19 knockdown using morpholino antisense oligonucleotides blocks AKT phosphorylation and activation. ADAM19 depletion does not greatly affect neural induction at the end of gastrulation, as seen by robust expression of *sox2*. At neural fold stage, however, *sox2* levels are increased in morphant embryos, while the emergence of the premigratory neural crest markers *snai2* and *sox8* is inhibited (Neuner et al., 2009, Fig. 3). In two-thirds of the tadpoles, morphant NCCs fail to migrate, although it remains unknown whether this phenotype is a consequence of the early defects in premigratory NC patterning or an independent effect of ADAM19 depletion on cell migration. Similar to ADAM19 depletion, the pharmacological blockade of EGF signaling or the broad-spectrum inhibition of ADAM metalloproteases blocks *snai2* activation in the premigratory NC in vivo (Neuner et al., 2009). These data demonstrate a novel function of EGF signaling in neural crest induction, likely mediated by AKT activity.

Direct evidence for a key function for AKT signaling in premigratory NC formation comes from two parallel studies, in fish and frog. Using either pharmacological inhibitors or inducible PFKFB4 depletion (using photoactivable morpholinos) PI3K/AKT signaling was blocked in a time-controlled manner during neurulation in order to avoid the effects on gastrula-stage embryos. In summary of the results described below, both series of experiments include a phenotype rescue by caAKT, demonstrating that in each case, altered AKT signaling is the major and specific cause of the observed premigratory neural crest defects (Ciarlo et al., 2017; Figueiredo et al., 2017, Fig. 3).

In zebrafish embryos, *crestin* is a neural crest-specific marker, activated by NB regulators and the late NC specifier *sox10* (Kaufman et al., 2016; Rubinstein et al., 2000). Ciarlo and colleagues have devised a novel high throughput cellular assay using *crestin-gfp* reporter zebrafish embryos dissociated into individual cells, to automate and screen the effects of pharmacological compounds on *crestin*-dependent *gfp* activation (Ciarlo et al., 2017). In this assay, the cells are grown in a medium containing EGF, insulin, and FGF, all three being able to activate AKT signaling. The authors find that caffeic acid phenethyl ester (CAPE), previously reported as an inhibitor of PI3K activity in melanoma cell lines (Pramanik et al., 2013), and several other PI3K inhibitors, decrease *crestin-gfp* appearance both in dissociated cells and in whole embryos. Embryos are treated at the 2-somite stage, i.e. a stage when the cephalic neural crest is already specified as known from the expression pattern of NB markers (*pax3),* early premigratory NC markers (*foxd3)* or the late premigratory NC markers (*sox10* and *twist1a*) (Dutta and Dawid, 2010; Germanguz et al., 2007). CAPE treatment at this stage is thus expected to affect either the maintenance of already specified cranial NC or the induction of posterior trunk NC, but not the formation of the neural plate or the neural border. Analysis of CAPE-treated embryos shows that indeed, NB formation (marked by *pax3a, msxb, tfap2a*, and *tfap2c* expression) is globally normal along the entire anterior-posterior axis, as well as early premigratory NC specification (from *snai1, snai2, foxd3*, and *ets1* expression) although some NB/NC markers display up to 30% decreased expression levels compared to controls. In particular, CAPE treatment causes a modest decrease in expression of the late NC marker *sox10* (17% less relative to control), mainly in trunk NC (Fig. 3). In contrast, downstream NC gene expression is defective in the premigratory trunk NC: *crestin* or tfap2-target *inka* are severely decreased, as are genes involved in pigment cell development (*mitfa, tyr, ednrba, fms)* (Fig. 5). Molecularly, both insulin and FGF synergize to activate *crestin-gfp* in the zebrafish cell cultures. While insulin signaling acts mostly via AKT activation, FGF signals can activate several pathways including AKT and MAPK (MEK-ERK) signaling as described in several paragraphs above. The phenotype of CAPE-treated embryos is rescued by caAKT, as shown by restored *crestin* expression, improved melanocyte induction and migration in vivo. This indicates that AKT activity is a major target of CAPE in NC formation. Importantly, in transgenic fish embryos with a constitutive activation of PTEN, both AKT phosphorylation and *crestin* expression are reduced, confirming the central function of AKT signaling in zebrafish NC (Ciarlo et al., 2017).

In *Xenopus laevis* embryos, PFKFB4 expression becomes spatially restricted to the premigratory NC during the neural fold stage, similar to *sox10*. This refinement appears to depend on the main NC-GRN regulators, e.g. *pax3, tfap2a,* and *sox9* (Figueiredo et al., 2017). PFKFB4-depleted NC displays reduced phospho-AKT levels while and caAKT rescues the PFKFB4 morphant phenotypes. In contrast, MEK/ERK signaling remains unaffected. As observed in CAPE-treated zebrafish, in conditions that bypass early neurula stages (either using low-level depletion with standard morpholinos or photo-inducible morpholinos), the main phenotype in the PFKFB4 morphants is the defective activation of a subset of late markers in the premigratory NC: *twist1* is severely diminished in 65% of the embryos, while *sox10* is decreased in 25% of the embryos. In parallel, expression of NB and immature NC markers (*pax3*, *zic1*, *hes4* and *cmyc*) are increased, indicating that the PFKFB4-AKT-defective NB cells generates a normal pool of premigratory NC stem cells, but these cells fail to complete their specification during neural fold stages. Direct inhibition of PI3K by LY2940042, during the neural fold stage results in the similar main-

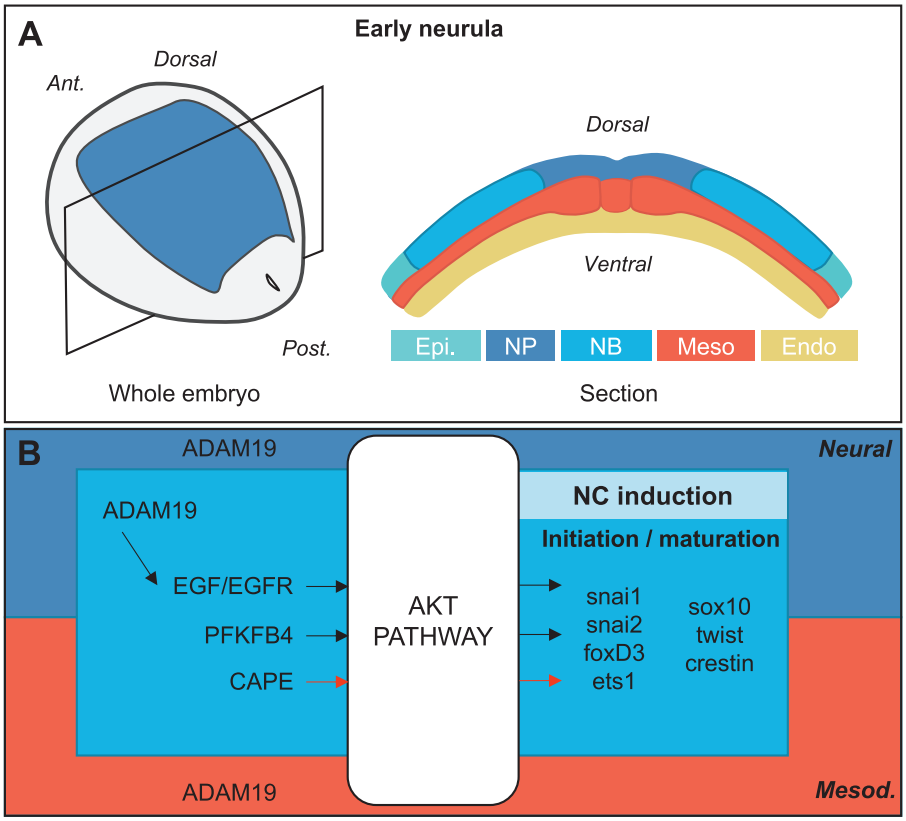

Fig. 3. Regulations linked to AKT signaling during neural crest induction. (A) Scheme of whole early frog neurula and cross section. Anterior (Ant.); Posterior (Post.); Mesoderm (Meso.); Endoderm (Endo.); Epiderm (Epi.); Neural plate (NP); Neural border (NB). (B) The regulations upstream of AKT signaling, involving either ADAM19-EGF signaling, PFKFB4 or the chemical component CAPE are listed. Downstream events during the first phase of NC induction (initiation) or the maturation of premigratory NC (maturation) are indicated, with the main NC markers for each stage used in the various studies.

tenance of the NC stem cell marker *c-myc* and blockade of *twist1* activation, while MAPK pathway inhibition (using UO126) has no effect. Together, the three different modes of AKT inhibition, by CAPE or LY294002, or PFKFB4 depletion, both in fish and frog embryos, highlight the direct function of PI3K-AKT signaling on the maturation of premigratory NC cells in late neurulas prior to EMT (Fig. 3).

3. AKT signaling modulates EMT and migration of the neural crest cells

The defining feature of NC cells is their delamination from the dorsal neural epithelium. At the end of neurulation, mature premigratory NC cells lose their epithelial organization, acquire a mesenchymal morphology and emigrate from the neural tube towards multiple locations in the embryo. NCC modify their cell-cell contacts and cell-substratum adhesion properties, mediated notably by changes in the cadherins located at the plasma membrane and by modulation of integrin signaling (Mayor and Theveneau, 2013; Nieto et al., 2016). The NCC cytoskeleton is actively remodeled by a dynamic regulation of the actin network, allowing flexibility and fast morphology changes while the rhythm of cell divisions becomes coordinated with the constraints of cell migration (Nieto et al., 2016). During NC EMT and migration, the AKT pathway is involved in at least three of these cellular modifications: cytoskeleton changes, the stability of cell-cell junctions, and the nature of cell-substratum interactions in mouse, fish and frog embryos.

3.1. AKT signaling modulates cell-cell and cell-matrix contacts upstream of EMT

Intercellular adherens junctions between cells involve cadherins connected to the actin cytoskeleton via catenins α and β and other junctional proteins (Aberle et al., 1996; Nelson, 2008). As NCC lose their epithelial structure, they still form transient cell-cell contacts, allowed by a switch from E-cadherin (cdh1) to other cadherins (e.g. N-cadherin, cadherin-6, or cadherin-11). Tightly regulated levels of these cadherins are essential for NCC migration, as 'moderate' levels of N-cadherin allow a balance between transitory cell contacts during collective migration, and cell dispersion by contact-inhibition of locomotion (CIL) (Mayor and Theveneau, 2013). AKT signaling is implicated in the transcriptional control of cadherin gene expression prior to EMT. In frog premigratory NCC, both PDGFRα-PDGFA signaling and PFKFB4 are required for EMT from the NB tissue both in vitro and in vivo, and regulate *n-cadherin* gene activation (Bahm et al., 2017; Figueiredo et al., 2017, Fig. 4). Moreover, PDGFRα-PDGFA activates *n-cadherin* in an AKT-dependent manner (Bahm et al., 2017).

In parallel, the AKT pathway is involved in the stability of the protein complexes forming the cell-cell junctions. Mice mutant for SPECC1L, a gene predicted to encode a coiled-coil domain protein interacting with the cytoskeleton (Saadi et al., 2011), exhibit a severe facial clefting phenotype, resulting from a dramatic alteration of craniofacial morphogenesis, a condition also seen with a lesser severity in human patients with heterozygous SPECC1L mutation (Wilson et al., 2016). Homozygous SPECC1L deficient mouse embryos present reduced NCC delamination from the cranial neural folds, increased premigratory NCC apoptosis, while the earlier premigratory neural crest patterning is normal (Wilson et al., 2016). Compared to wild-type NCC, SPECC1L mutant NCC morphology is altered, with more numerous actin stress fibers and stabilized adherens junctions enriched in β-catenin and E-cadherin (cdh1), both in cell culture and at the edges of the neural folds in vivo. SPECC1L deficiency causes a decrease

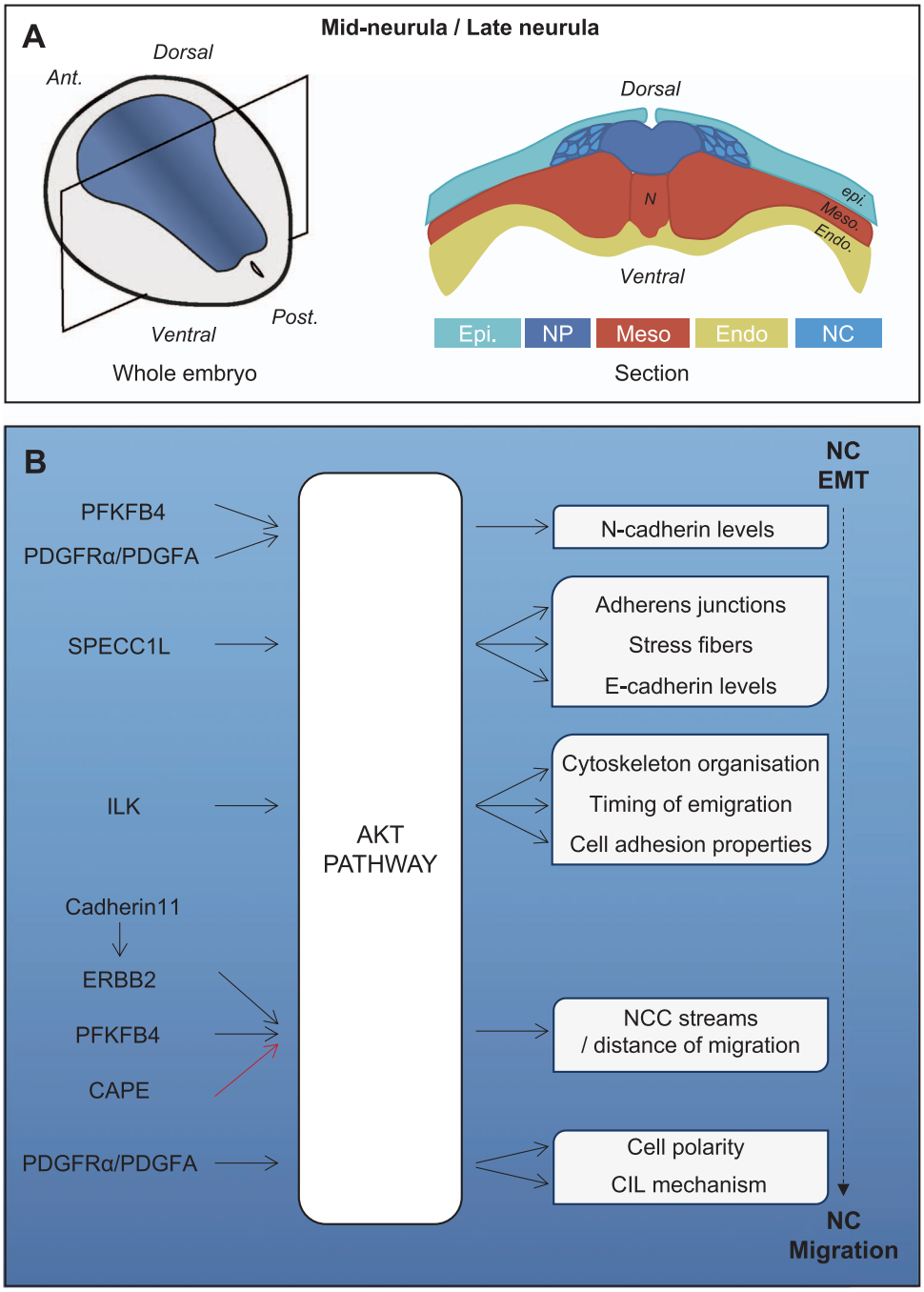

Fig. 4. Regulations linked to AKT signaling during neural crest EMT and migration. (A) Scheme of whole late frog neurula and cross section. Anterior (Ant.); Posterior (Post.); Mesoderm (Meso.); Endoderm (Endo.); Epiderm (Epi.); Neural plate (NP); Neural crest (NC); Notochord (N). (B) The regulations controlling AKT signaling impacting the different parameters of NC EMT and migration are indicated, including early regulation of cell-cell and cell-substrate junctions, cytoskeleton organization, and parameters of cell motility and migration. Red arrows indicate negative regulations.

of total AKT and of phospho-AKT levels. Blocking AKT in normal cells mimics the mutant cytoskeleton phenotype, while the pharmacological reactivation of AKT activity in SPECC1L deficient cells restores normal cellular morphology in vitro. While a direct link between SPECC1L and AKT regulation remains unclear, this study demonstrates that adequate NCC emigration from the neural folds relies on the AKT-dependent destabilization of adherens junctions (Wilson et al., 2016).

In addition to cell-cell adhesion regulation, AKT mediates integrin signaling to regulate cell-matrix contacts and the timing of EMT. In mouse embryos, NC-specific deletion of integrin-linked kinase (ILK), a key component of focal adhesions (Legate et al., 2006), leads to the premature emigration of NCC, followed by altered migration. ILK-deficient NC present decreased phospho-AKT levels, disorganized cytoskeleton and focal adhesion foci, together with altered cell adhesion properties on several components of the extracellular matrix (Dai et al., 2013, Fig. 4).

Altogether, these studies highlight the pivotal role of AKT signals in the multiple cellular transformations of NCC involved in their spatially

and temporally coordinated exit from the dorsal neural tube.

3.2. AKT signaling controls neural crest cell migration

After EMT, NCC migrate along defined routes in the embryo, under multiple controls including cell-cell guidance (collective migration, CIL), attractant and repulsive cues (Carmona-Fontaine et al., 2011; Gammill et al., 2006; Theveneau and Mayor, 2014). The modulation of these parameters impacts the velocity, the directionality and the distance of migration (Carmona-Fontaine et al., 2008). Several of these parameters are controlled by AKT signaling. In addition to examples of AKT regulation by various actors already described above, the cleavage of cadherin-11 produces a soluble extracellular fragment, EC1-3, which bind EGF receptor ERBB2 and stimulates AKT phosphorylation in NCC in vivo (Mathavan et al., 2017, Fig. 4). Studies conducted both in frog and in fish embryos show that either directly blocking AKT signaling, or indirectly preventing AKT activation (by inhibition of PI3K, knockdown of PFKFB4, CAPE treatment, or blocking EGF signaling), decreases the size of NCC streams and their distance of migration. CAPE-treated NCC fail to reach the ventral trunk of the embryo: the cells maintain a round morphology and do not make cytoplasmic extensions as usually seen in migrating cells. As a consequence, body pigmentation is significantly reduced while melanocytes remain clustered dorsal to the neural tube (Ciarlo et al., 2017). While most in vivo studies have used treatments encompassing both premigratory NC maturation at neural fold stage, EMT and migration (Bahm et al., 2017; Ciarlo et al., 2017; Mathavan et al., 2017), NC migration is also inhibited when PFKFB4 knockdown or PI3K blockade are done selectively after EMT of the cranial NCC and during their initial stages of migration (Figueiredo et al., 2017). The detailed analysis of PDGF signal activation on frog premigratory NCC in vitro shows that the PDGFA ligand induces the rapid accumulation of AKT at the plasma membrane and AKT phosphorylation. Such AKT activation in response to PDGFR signaling is accompanied by the acquisition of cell polarity, migration and dispersion as a mark of CIL (Bahm et al., 2017, Fig. 4). As a whole, these studies demonstrate that defective AKT signaling affects NC migration, resulting in a limited population of NCC reaching their target tissues, thus potentially resulting in the formation of NC-derived organs reduced in size. According to this prediction, mice with a targeted PDGFRα mutation in the neural crest ($Pdgfr\alpha^{flox/flox}$; Wnt1-cre) have a reduced number of cells reaching the craniofacial prominences (He and Soriano, 2013). In vitro, those cells exhibited altered cell-substratum adhesion and abnormal lamellipodia, confirming the defective migratory abilities (He and Soriano, 2013).

4. AKT signaling promotes the differentiation of several NCC-derived lineages

In several instances, such as the differentiation of the craniofacial skeleton and the peripheral nervous system, NCC differentiation depends on AKT signaling. Because many growth factor receptors simultaneously activate multiple downstream signaling cascades, we here focus on regulators such as PTEN that have been formally linked to PI3K-AKT.

4.1. The proliferation and survival of peripheral neurons are stimulated by AKT signaling

The enteric nervous system is formed by NC-derived neurons and glia which are essential for intestine motility. These emerge mainly from the vagal level and undergo a long-distance migration along the gut, populating the myenteric and the submucosal plexuses (Burns and Le Douarin, 1998). Cell migration and gut colonization are coordinated with extensive cell proliferation prior to differentiation. ENS development, including migration, proliferation, survival and maturation, is largely controlled by RET tyrosine kinase receptor-dependent signaling (Airaksinen and Saarma, 2002; Jiang et al., 2015, Fig. 5). In addition, Ret deficient mice also present defects in the other peripheral neurons in the sympathetic, autonomous and sensory lineages (Schuchardt et al., 1994). RET activation triggers both MAPK and PI3K-AKT intracellular pathways (Santoro et al., 1994; van Weering and Bos, 1997). Specifically, GDNF treatment of ENS neurons, in culture, promotes AKT-dependent neuronal survival and neurite extension while MAPK does not seem involved (Srinivasan et al., 2005). Enteric neuron proliferation, in contrast, is activated both by AKT and MAPK signaling (Ngan et al., 2007). Similarly, the trigeminal ganglia and the maxillary nerves are enlarged in mice mutant for PTEN, due to an increased proliferation rate caused by enhanced AKT signaling (Yang et al., 2017, Fig. 5).

4.2. PI3K-AKT signals stimulate proliferation and differentiation of skeletal derivatives

Craniosynostosis is characterized by the premature fusion of the calvaria (skull bones), most of which are derived from the neural crest (Couly et al., 1993). Craniosynostosis is a frequent human birth defect, arising in 1/2500 births. It can be caused by mutations in genes encoding either receptor tyrosine kinases (RTK), or their ligands or a downstream signaling component (Johnson and Wilkie, 2011). For example, mutations in FGFR2 and 3 lead to Apert and Crouzon syndromes which are associated with craniofacial defects and craniosynostosis in human (Johnson and Wilkie, 2011). When targeted to the neural crest lineage in mouse embryos, FGFR2/3 mutations reproduce the craniosynostosis phenotype (Su et al., 2014). Although FGFRs potentially activate either MAPK, PI3K-AKT or PLC γ signaling, MAPK signaling is the major mediator of FGFRs function in the NC-derived mouse skeleton (Matsushita et al., 2009; Shukla et al., 2007). PDGF receptors are another class of RTKs, essential for NC development, involved in human craniosynostosis, and engaging multiple intracellular signaling such as MAPK, Rac1, SRC, PLC γ or PI3K/AKT (He and Soriano, 2013; Klinghoffer et al., 2001; Miraoui et al., 2010; Soriano, 1997; Tallquist and Soriano, 2003; Vasudevan et al., 2015). In mice, the ubiquitous expression of a constitutively active form of PDGFRα leads to craniosynostosis, caused by simultaneous defects in the neural crest-derived and the mesoderm-derived skeletal cells (He and Soriano, 2017; Moenning et al., 2009). The differentiation of both mesoderm-derived chondroblasts underlying the coronal suture, and of NC-derived interfrontal, sagittal and coronal suture osteoblasts is enhanced and accelerated when PDGFRα signaling is increased. Molecularly, activating PDGFRα signaling in vivo, either with caPDGFRα or by inhibiting PTEN, increases PLC γ and AKT phosphorylation, while SRC, ERK and STAT3 remained unchanged (He and Soriano, 2017; Moenning et al., 2009). Conversely, mouse mutants with a PDGFRα allele unable to activate PI3K signaling show delayed suture ossification and hypoplastic cartilage (Yang et al., 2017; Fig. 5). Other types of NC-derived skeletogenic mesenchyme are regulated in a similar manner. For example, the osteogenic differentiation of the frontonasal mesenchyme, grown in primary culture, is stimulated by PDGFRα in an AKT-dependent mechanism (Vasudevan et al., 2015). Similarly, primary mesenchyme cells dissected from the premaxilla of E13.5 PTEN KO mice show increased cell proliferation and elevated p-AKT levels in primary culture. The PI3K inhibitor LY294002 suppresses both overproliferation and alkaline phosphatase activity in those cells. In contrast, premaxillary mesenchyme with a mutant allele of PDGFRα unable to trigger PI3K presents reduced proliferation and differentiation (Yang et al., 2017). In vivo, the subcellular localization of all actors of the AKT signaling cascade is tightly regulated. For example, PDGF receptors are enriched in the primary cilium of neural crest cells. Mice with mutations in the intraflagellar transport protein IFT20 have altered ciliogenesis and reduced PDGFR expression at the cell surface. When this mutation is targeted to the neural crest ($ift20^{flox/flox}$:*Wnt1-cre* mice) the mice present defective facial bone differentiation (severe osteopenia). On the one hand, this phenotype is caused by decreased osteoblast cell proliferation and increased apoptosis, while on the other hand, there appears to be an AKT-independent mineralization defect of skull bones

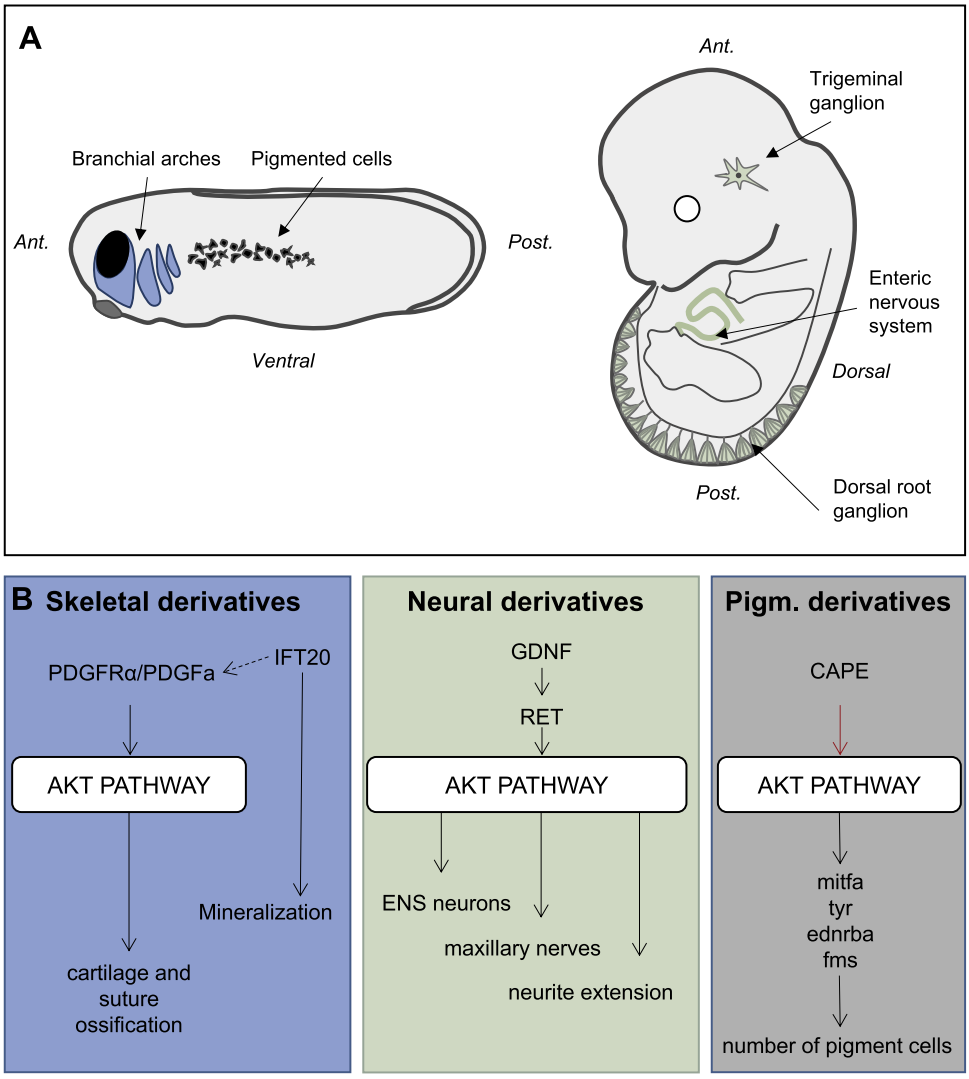

Fig. 5. Regulations linked to AKT signaling during neural crest differentiation. (A) NC differentiation into different cell types, indicated in the schematics has been studied in frog, fish and mouse embryos. Anterior (Ant.); Posterior (Post.); Enteric nervous system (ENS). (B) The regulations impacting AKT signaling and their outcome on NC differentiation are indicated for bone and cartilage, neurons and pigment cells derived from the neural crest.

(Noda et al., 2016). Altogether, these studies demonstrate the critical impact of the PI3K-AKT pathway to promote the proliferation, survival and differentiation of several NC-derived cell types, including neuron progenitors, chondroblasts and osteoblasts, at various stages of embryogenesis (Fig. 5).

5. Conclusions and perspectives

Recent studies highlight that the AKT signaling pathway is involved at all the steps of neural crest development, from establishment of the dorsal ectoderm and mesoderm during gastrulation to ectoderm regionalization and patterning of the neural border as well as for induction and maturation of the premigratory neural crest. AKT appears to play key cellular roles during these stages, including promoting EMT and migration, as well as for cell proliferation and survival during the differentiation of the neural crest derivatives. Because of this broad requirement for AKT activity, the experimental definition of AKT function at each individual step, in vivo, has required the use of time-controlled modulation of AKT signaling, e.g. using pharmacological agents, as well as by the use of tissue-specific spatial control of AKT activity with targeted strategies. In the developing embryo, the spatial and temporal dynamics of selected ligands and receptors orchestrates the fine-tuned activity of AKT. Specifically, in neural crest progenitors or tissues related to neural crest development, AKT signaling is activated and sustained in a tissue and time-specific manner by ligands binding RTKs or by non-conventional regulators, linked to the NC-GRN. For example, PDGFRα and PFKFB4 expression are controlled by NB/NC regulators (Figueiredo et al., 2017; Plouhinec et al., 2014). Collectively, these studies demonstrate that in the neural crest as in other cellular systems, AKT signaling is a hub potentially integrating the inputs from multiple pathways, and translating them into a coordinated cellular response, such as cell proliferation, survival, migration, or differentiation. In the neural crest context, it remains largely unknown which AKT effector(s) is (are) engaged to elicit each response. Comparison with adult cells or cancer cells with altered AKT response, which have been extensively studied could help us find the relevant effectors: for example, *snai2*, an essential transcription factor for neural crest induction and EMT initiation, is involved in migration and EMT of cancer such as melanoma, carcinoma or thyroid cancer (Fenouille et al., 2012; Julien et al., 2007; Visciano et al., 2015). Moreover, AKT is described to use TWIST1 to regulate cell migration in breast cancer and provide antiapoptotic properties in nasopharyngeal carcinoma cells (Li and Zhou, 2011; Zhang et al., 2007). Other potential AKT effectors from cancer studies, not yet recorded to participate in NC development could be identified by checking their expression at the different stages of NC formation in large-scale

transcriptome studies (Plouhinec et al., 2017; Rabadán et al., 2013).

Many of the cellular processes regulated by AKT activation are also controlled by other intracellular pathways such as the MAPK pathway, the PLCγ pathway, but also, directly or indirectly, by signaling pathways not linked to RTK transmembrane receptors, such as growth factor-linked signaling affecting important steps of neural crest formation, e.g. BMPs or Wnt signals. The complex crosstalk between pathways, its temporal, spatial and cell-type specificity, as well as the importance of the temporal dynamics of each signal, are emerging as key parameters necessary to elicit the appropriate response in each cell context (Ciarlo et al., 2017; Vasudevan et al., 2015). Additional modulation of AKT activation by posttranslational modifications, redundancy or specific roles for each member of the families of kinases, phosphatases, and other enzymes involved in AKT modifications, adds a further layer of biological complexity to the matter. Altogether, the recent involvement of AKT signaling at all the steps of neural crest formation for multiple responses, including early patterning events which were thought to rely mainly on transcriptional regulation, implicate this pathway as an integral partner of the NC-GRN in future studies.

Acknowledgements and funding

The authors are very grateful to Dr. K. Liu and P. Scerbo for their critical reading of the manuscript, and to all members of the Monsoro-Burq team for interactive discussions. This study was supported by funding from Université Paris Sud, Centre National de la Recherche Scientifique (CNRS), Agence Nationale pour la Recherche (ANR Programme Blanc CrestNetMetabo: ANR-15-CE13-0012-01-CRESTNETMETABO), and Fondation pour la Recherche Médicale (FRM, Programme Equipes Labellisées DEQ20150331733) and Institut Universitaire de France to AHMB. M.S. is a Ph.D. fellow funded by Fondation pour la Recherche Médicale (ECO20160736105).

References

Aberle, H., Schwartz, H., Kemler, R., 1996. Cadherin-catenin complex: protein interactions and their implications for cadherin function. J. Cell. Biochem. 61, 514–523. http://dx.doi.org/10.1002/(SICI)1097-4644(19960616)61:4<514::AID-JCB4>3.0.CO;2-R.

Airaksinen, M.S., Saarma, M., 2002. The GDNF family: signalling, biological functions and therapeutic value. Nat. Rev. Neurosci. 3, 383–394. http://dx.doi.org/10.1038/nrn812.

Alessi, D.R., Andjelkovic, M., Caudwell, B., Cron, P., Morrice, N., Cohen, P., Hemmings, B.A., 1996. Mechanism of activation of protein kinase B by insulin and IGF-1. EMBO J. 15, 6541–6551.

Alessi, D.R., Deak, M., Casamayor, A., Caudwell, F.B., Morrice, N., Norman, D.G., Gaffney, P., Reese, C.B., MacDougall, C.N., Harbison, D., Ashworth, A., Bownes, M., 1997. 3-Phosphoinositide-dependent protein kinase-1 (PDK1): structural and functional homology with the Drosophila DSTPK61 kinase. Curr. Biol. 7, 776–789.

Amaya, E., Stein, P.A., Musci, T.J., Kirschner, M.W., 1993. FGF signalling in the early specification of mesoderm in Xenopus. Development, 118.

Aybar, M.J., Mayor, R., 2002. Early induction of neural crest cells: lessons learned from frog, fish and chick. Curr. Opin. Genet. Dev. 12, 452–458. http://dx.doi.org/10.1016/S0959-437X(02)00325-8.

Bahm, I., Barriga, E.H., Frolov, A., Theveneau, E., Frankel, P., Mayor, R., 2017. PDGF controls contact inhibition of locomotion by regulating N-cadherin during neural crest migration. (dev.147926)Development 144. http://dx.doi.org/10.1242/dev.147926.

Basch, M.L., Bronner-Fraser, M., 2006. Neural crest inducing signals. Adv. Exp. Med. Biol.. http://dx.doi.org/10.1007/978-0-387-46954-6_2.

Bronner, M., LeDouarin, N., 2012. Evolution and development of the neural crest: an overview. Dev. Biol. 366, 2–9. http://dx.doi.org/10.1016/j.ydbio.2011.12.042.Evolution.

Burns, A.J., Le Douarin, N.M., 1998. The sacral neural crest contributes neurons and glia to the post-umbilical gut: spatiotemporal analysis of the development of the enteric nervous system. Development, 125.

Carballada, R., Yasuo, H., Lemaire, P., 2001. Phosphatidylinositol-3 kinase acts in parallel to the ERK MAP kinase in the FGF pathway during Xenopus mesoderm induction. Development 128, 35–44.

Carmona-Fontaine, C., Matthews, H.K., Kuriyama, S., Moreno, M., Dunn, G.A., Parsons, M., Stern, C.D., Mayor, R., 2008. Contact inhibition of locomotion in vivo controls neural crest directional migration. Nature 456, 957–961. http://dx.doi.org/10.1038/nature07441.

Carmona-Fontaine, C., Theveneau, E., Tzekou, A., Tada, M., Woods, M., Page, K.M., Parsons, M., Lambris, J.D., Mayor, R., 2011. Complement fragment C3a controls mutual cell attraction during collective cell migration. Dev. Cell 21, 1026–1037. http://dx.doi.org/10.1016/J.DEVCEL.2011.10.012.

Ciarlo, C., Kaufman, C.K., Kinikoglu, B., Michael, J., Yang, S., D'Amato, C., Blokzijl-Franke, S., den Hertog, J., Schlaeger, T., Zhou, Y., Liao, E.C., Zon, L.I., 2017. A chemical screen in zebrafish embryonic cells establishes that Akt activation is required for neural crest development. Elife. http://dx.doi.org/10.7554/eLife.29145.

Couly, G.F., Coltey, P.M., Le Douarin, N.M., 1993. The triple origin of skull in higher vertebrates: a study in quail-chick chimeras. Development, 117.

Dai, X., Jiang, W., Zhang, Q., Xu, L., Geng, P., Zhuang, S., Petrich, B.G., Jiang, C., Peng, L., Bhattacharya, S., Evans, S.M., Sun, Y., Chen, J., Liang, X., 2013. Requirement for integrin-linked kinase in neural crest migration and differentiation and outflow tract morphogenesis. BMC Biol. 11, 107. http://dx.doi.org/10.1186/1741-7007-11-107.

de Crozé, N., Maczkowiak, F., Monsoro-Burq, A.H., 2011. Reiterative AP2a activity controls sequential steps in the neural crest gene regulatory network. Proc. Natl. Acad. Sci. USA 108, 155–160. http://dx.doi.org/10.1073/pnas.1010740107.

Divecha, N., Irvine, R.F., 1995. Phospholipid signaling. Cell 80, 269–278. http://dx.doi.org/10.1016/0092-8674(95)90409-3.

Dutta, S., Dawid, I.B., 2010. Kctd15 inhibits neural crest formation by attenuating Wnt/beta-catenin signaling output. Development 137, 3013–3018. http://dx.doi.org/10.1242/dev.047548.

Faure, S., De Santa Barbara, P., Roberts, D.J., Whitman, M., 2002. Endogenous patterns of BMP signaling during early chick development. Dev. Biol. 244, 44–65. http://dx.doi.org/10.1006/dbio.2002.0579.

Fenouille, N., Tichet, M., Dufies, M., Pottier, A., Mogha, A., Soo, J.K., Rocchi, S., Mallavialle, A., Galibert, M.-D., Khammari, A., Lacour, J.-P., Ballotti, R., Deckert, M., Tartare-Deckert, S., 2012. The epithelial-mesenchymal transition (EMT) regulatory factor SLUG (SNAI2) is a downstream target of SPARC and AKT in promoting melanoma cell invasion. PLoS One 7, e40378. http://dx.doi.org/10.1371/journal.pone.0040378.

Figueiredo, A.L., Maczkowiak, F., Borday, C., Pla, P., Sittewelle, M., Pegoraro, C., Monsoro-burq, A.H., 2017. PFKFB4 control of Akt signaling is essential for premigratory and migratory neural crest formation. Development. http://dx.doi.org/10.1242/dev.157644.

Gammill, L.S., Gonzalez, C., Gu, C., Bronner-Fraser, M., 2006. Guidance of trunk neural crest migration requires neuropilin 2/semaphorin 3F signaling. Development 133, 99–106. http://dx.doi.org/10.1242/dev.02187.

Garcia-Castro, M.I., Marcelle, C., Bronner-Fraser, M., 2002. Ectodermal Wnt function as a neural crest inducer. (80-.)Science 297, 848–851. http://dx.doi.org/10.1126/science.1070824.

Garnett, A.T., Square, T.A., Medeiros, D.M., 2012. BMP, Wnt and FGF signals are integrated through evolutionarily conserved enhancers to achieve robust expression of Pax3 and Zic genes at the zebrafish neural plate border. Development 139, 4220–4231. http://dx.doi.org/10.1242/dev.081497.

Germanguz, I., Lev, D., Waisman, T., Kim, C.-H., Gitelman, I., 2007. Four twist genes in zebrafish, four expression patterns. Dev. Dyn. 236, 2615–2626. http://dx.doi.org/10.1002/dvdy.21267.

Glinka, A., Wu, W., Delius, H., Monaghan, A.P., Blumenstock, C., Niehrs, C., 1998. Dickkopf-1 is a member of a new family of secreted proteins and functions in head induction. Nature 391, 357–362. http://dx.doi.org/10.1038/34848.

Harland, R., 2000. Neural induction. Methods Cell Biol. 36, 329–346.

He, F., Soriano, P., 2017. Dysregulated PDGFRα signaling alters coronal suture morphogenesis and leads to craniosynostosis through endochondral ossification. Dev. Dev., 151068. http://dx.doi.org/10.1242/dev.151068.

He, F., Soriano, P., 2013. A critical role for PDGFRα signaling in medial nasal process development. PLoS Genet., 9. http://dx.doi.org/10.1371/journal.pgen.1003851.

Hers, I., Vincent, E.E., Tavaré, J.M., 2011. Akt signalling in health and disease. Cell Signal. 23, 1515–1527. http://dx.doi.org/10.1016/j.cellsig.2011.05.004.

Hindley, C.J., Condurat, A.L., Menon, V., Thomas, R., Azmitia, L.M., Davis, J.A., Pruszak, J., 2016. The Hippo pathway member YAP enhances human neural crest cell fate and migration. Sci. Rep. 6, 23208. http://dx.doi.org/10.1038/srep23208.

Horiuchi, K., Zhou, H.M., Kelly, K., Manova, K., Blobel, C.P., 2005. Evaluation of the contributions of ADAMs 9, 12, 15, 17, and 19 to heart development and ectodomain shedding of neuregulins β1 and β2. Dev. Biol. 283, 459–471. http://dx.doi.org/10.1016/j.ydbio.2005.05.004.

Jiang, Q., Arnold, S., Heanue, T., Kilambi, K.P., Doan, B., Kapoor, A., Ling, A.Y., Sosa, M.X., Guy, M., Jiang, Q., Burzynski, G., West, K., Bessling, S., Griseri, P., Amiel, J., Fernandez, R.M., Verheij, J.B.G.M., Hofstra, R.M.W., Borrego, S., Lyonnet, S., Ceccherini, I., Gray, J.J., Pachnis, V., McCallion, A.S., Chakravarti, A., 2015. Functional loss of semaphorin 3C and/or semaphorin 3D and their epistatic interaction with ret are critical to hirschsprung disease liability. Am. J. Hum. Genet. 96, 581–596. http://dx.doi.org/10.1016/j.ajhg.2015.02.014.

Johnson, D., Wilkie, A.O.M., 2011. Craniosynostosis. Eur. J. Hum. Genet. 19, 369–376. http://dx.doi.org/10.1038/ejhg.2010.235.

Julien, S., Puig, I., Caretti, E., Bonaventure, J., Nelles, L., van Roy, F., Dargemont, C., de Herreros, A.G., Bellacosa, A., Larue, L., 2007. Activation of NF-κB by Akt upregulates snail expression and induces epithelium mesenchyme transition. Oncogene 26, 7445–7456. http://dx.doi.org/10.1038/sj.onc.1210546.

Kaufman, C.K., Mosimann, C., Fan, Z.P., Yang, S., Thomas, A.J., Ablain, J., Tan, J.L., Fogley, R.D., van Rooijen, E., Hagedorn, E.J., Ciarlo, C., White, R.M., Matos, D.A., Puller, A.C., Santoriello, C., Liao, E.C., Young, R.A., Zon, L.I., Rooijen, E., Van, Ciarlo, C., White, R.M., Matos, D.A., Puller, A.C., 2016. A zebrafish melanoma model reveals emergence of neural crest identity during melanoma initiation. (aad2197, 80-.)Science 351. http://dx.doi.org/10.1126/science.aad2197.

Kirby, M.L., Hutson, M.R., 2010. Factors controlling cardiac neural crest cell migration. Cell Adhes. Migr. 4, 609–621. http://dx.doi.org/10.4161/cam.4.4.13489.

Klinghoffer, R.A., Mueting-Nelsen, P.F., Faerman, A., Shani, M., Soriano, P., 2001. The two PDGF receptors maintain conserved signaling in vivo despite divergent embryological functions. Mol. Cell 7, 343–354. http://dx.doi.org/10.1016/S1097-2765(01)00182-4.

Kurohara, K., Komatsu, K., Kurisaki, T., Masuda, A., Irie, N., Asano, M., Sudo, K., Nabeshima, Y.I., Iwakura, Y., Sehara-Fujisawa, A., 2004. Essential roles of meltrin β (ADAM19) in heart development. Dev. Biol. 267, 14–28. http://dx.doi.org/10.1016/j.ydbio.2003.10.021.

Larabell, C.A., Torres, M., Rowning, B.A., Yost, C., Miller, J.R., Wu, M., Kimelman, D., Moon, R.T., 1997. Establishment of the dorso-ventral axis in Xenopus embryos is presaged by early asymmetries in beta-catenin that are modulated by the Wnt signaling pathway. J. Cell Biol. 136, 1123–1136.

Legate, K.R., Montañez, E., Kudlacek, O., Fässler, R., 2006. ILK, PINCH and parvin: the tIPP of integrin signalling. Nat. Rev. Mol. Cell Biol.. http://dx.doi.org/10.1038/nrm1789.

Li, J., Zhou, B.P., 2011. Activation of β-catenin and Akt pathways by twist are critical for the maintenance of EMT associated cancer stem cell-like characters. BMC Cancer 11, 49. http://dx.doi.org/10.1186/1471-2407-11-49.

Ma, W., Sung, H.J., Park, J.Y., Matoba, S., Hwang, P.M., 2007. A pivotal role for p53: balancing aerobic respiration and glycolysis. J. Bioenerg. Biomembr. 39, 243–246. http://dx.doi.org/10.1007/s10863-007-9083-0.

McMahon, A.P., Moon, R.T., 1989. Ectopic expression of the proto-oncogene int-1 in Xenopus embryos leads to duplication of the embryonic axis. Cell 58, 1075–1084.

Maehama, T., Dixon, J.E., 1998. The tumor suppressor, PTEN/ MMAC1, dephosphorylates the lipid second messenger, phosphatidylinositol 3,4,5-trisphosphate. J. Biol. Chem. 273, 13375–13378.

Manning, B.D., Cantley, L.C., 2007. AKT/PKB signaling: navigating downstream. Cell. http://dx.doi.org/10.1016/j.cell.2007.06.009.

Manning, B.D., Toker, A., 2017. AKT/PKB signaling: navigating the network. Cell 169, 381–405. http://dx.doi.org/10.1016/j.cell.2017.04.001.

Mathavan, K., Khedgikar, V., Bartolo, V., Alfandari, D., 2017. The ectodomain of cadherin-11 binds to erbB2 and stimulates Akt phosphorylation to promote cranial neural crest cell migration. Res. Art., 1–21.

Matsushita, T., Wilcox, W.R., Chan, Y.Y., Kawanami, A., Bükülmez, H., Balmes, G., Krejci, P., Mekikian, P.B., Otani, K., Yamaura, I., Warman, M.L., Givol, D., Murakami, S., 2009. FGFR3 promotes synchondrosis closure and fusion of ossification centers through the MAPK pathway. Hum. Mol. Genet. 18, 227–240. http://dx.doi.org/10.1093/hmg/ddn339.

Mayor, R., Theveneau, E., 2013. The neural crest. Development 140, 2247–2251. http://dx.doi.org/10.1242/dev.091751.

Milet, C., Monsoro-Burq, A.H., 2012. Neural crest induction at the neural plate border in vertebrates. Dev. Biol. 366, 22–33. http://dx.doi.org/10.1016/j.ydbio.2012.01.013.

Mir, A., Kofron, M., Zorn, A.M., Bajzer, M., Haque, M., Heasman, J., Wylie, C.C., 2007. FoxI1e activates ectoderm formation and controls cell position in the Xenopus blastula. Development 134, 779–788. http://dx.doi.org/10.1242/dev.02768.

Miraoui, H., Ringe, J., Häupl, T., Marie, P.J., 2010. Increased EFG- and PDGFalpha-receptor signaling by mutant FGF-receptor 2 contributes to osteoblast dysfunction in Apert craniosynostosis. Hum. Mol. Genet. 19, 1678–1689. http://dx.doi.org/10.1093/hmg/ddq045.

Mizuseki, K., Kishi, M., Shiota, K., Nakanishi, S., Sasai, Y., 1998. SoxD: an essential mediator of induction of anterior neural tissues in Xenopus embryos. Neuron 21, 77–85. http://dx.doi.org/10.1016/S0896-6273(00)80516-4.

Moenning, A., Jäger, R., Egert, A., Kress, W., Wardelmann, E., Schorle, H., 2009. Sustained platelet-derived growth factor receptor alpha signaling in osteoblasts results in craniosynostosis by overactivating the phospholipase C-gamma pathway. Mol. Cell. Biol. 29, 881–891. http://dx.doi.org/10.1128/MCB.00885-08.

Monsoro-Burq, A.H., Wang, E., Harland, R., 2005. Msx1 and Pax3 cooperate to mediate FGF8 and WNT signals during Xenopus neural crest induction. Dev. Cell 8, 167–178 . http://dx.doi.org/10.1016/j.devcel.2004.12.017.

Moroishi, T., Hansen, C.G., Guan, K.-L., 2015. The emerging roles of YAP and TAZ in cancer. Nat. Rev. Cancer, 15. http://dx.doi.org/10.1038/nrc3876.

Nelson, W.J., 2008. Regulation of cell-cell adhesion by the cadherin-catenin complex. Biochem. Soc. Trans. 36, 149–155. http://dx.doi.org/10.1042/BST0360149.

Neuner, R., Cousin, H., McCusker, C., Coyne, M., Alfandari, D., 2009. Xenopus ADAM19 is involved in neural, neural crest and muscle development. Mech. Dev. 126, 240–255. http://dx.doi.org/10.1016/j.mod.2008.10.010.

Ngan, E.S.W., Lee, K.Y., Sit, F.Y.L., Poon, H.C., Chan, J.K.Y., Sham, M.H., Lui, V.C.H., Tam, P.K.H., 2007. Prokineticin-1 modulates proliferation and differentiation of enteric neural crest cells. Biochim. Biophys. Acta – Mol. Cell Res. 1773, 536–545. http://dx.doi.org/10.1016/j.bbamcr.2007.01.013.

Nichane, M., de Crozé, N., Ren, X., Souopgui, J., Monsoro-Burq, A.H., Bellefroid, E.J., 2008a. Hairy2-Id3 interactions play an essential role in Xenopus neural crest progenitor specification. Dev. Biol. 322, 355–367. http://dx.doi.org/10.1016/j.ydbio.2008.08.003.

Nichane, M., Ren, X., Souopgui, J., Bellefroid, E.J., 2008b. Hairy2 functions through both DNA-binding and non DNA-binding mechanisms at the neural plate border in Xenopus. Dev. Biol. 322, 368–380. http://dx.doi.org/10.1016/j.ydbio.2008.07.026.

Nie, S., Chang, C., 2007a. Regulation of Xenopus gastrulation by ErbB signaling. Dev. Biol. 303, 93–107. http://dx.doi.org/10.1016/j.ydbio.2006.10.039.

Nie, S., Chang, C., 2007b. PI3K and Erk MAPK mediate ErbB signaling in Xenopus gastrulation. Mech. Dev. 124, 657–667. http://dx.doi.org/10.1016/j.mod.2007.07.005.

Nieto, M.A., Huang, R.Y.Y.J., Jackson, R.A.A., Thiery, J.P.P., 2016. Emt: 2016. Cell 166, 21–45. http://dx.doi.org/10.1016/j.cell.2016.06.028.

Nikitina, N.V., Bronner-Fraser, M., 2009. Gene regulatory networks that control the specification of neural-crest cells in the lamprey. Biochim. Biophys. Acta – Gene Regul. Mech. 1789, 274–278. http://dx.doi.org/10.1016/j.bbagrm.2008.03.006.

Noda, K., Kitami, M., Kitami, K., Kaku, M., Komatsu, Y., 2016. Canonical and noncanonical intraflagellar transport regulates craniofacial skeletal development. Proc. Natl. Acad. Sci. USA 113, E2589–E2597. http://dx.doi.org/10.1073/pnas.1519458113.

O'Reilly, M.-A.J., Smith, J.C., Cunliffe, V., 1995. Patterning of the mesoderm in Xenopus: dose-dependent and synergistic effects of Brachyury and Pintallavis. Development.

Pegoraro, C., Figueiredo, A.L., Maczkowiak, F., Pouponnot, C., Eychene, A., Monsoro-Burq, A.H., 2015. PFKFB4 controls embryonic patterning via Akt signalling independently of glycolysis. Nat. Commun. 6, 5953. http://dx.doi.org/10.1038/ncomms6953.

Pegoraro, C., Monsoro-Burq, A.H., 2013. Signaling and transcriptional regulation in neural crest specification and migration: lessons from xenopus embryos. Wiley Interdiscip. Rev. Dev. Biol. 2, 247–259. http://dx.doi.org/10.1002/wdev.76.

Peng, Y., Jiang, B.-H., Yang, P.-H., Cao, Z., Shi, X., Lin, M.C.M., He, M.-L., Kung, H., 2004. Phosphatidylinositol 3-kinase signaling is involved in neurogenesis during *Xenopus* embryonic development. J. Biol. Chem. 279, 28509–28514. http://dx.doi.org/10.1074/jbc.M402294200.

Piccolo, S., Dupont, S., Cordenonsi, M., 2014. The biology of YAP/TAZ: hippo signaling and beyond. Physiol. Rev. 94, 1287–1312. http://dx.doi.org/10.1152/physrev.00005.2014.

Pla, P., Monsoro-burq, A.H., 2018. The neural border: induction, specification and maturation of the territory that generates Neural Crest cells. Dev. Biol.. http://dx.doi.org/10.1016/j.ydbio.2018.05.018.

Plouhinec, J.-L., Medina-Ruiz, S., Borday, C., Bernard, E., Vert, J.-P., Eisen, M.B., Harland, R.M., Monsoro-Burq, A.H., 2017. A molecular atlas of the developing ectoderm defines neural, neural crest, placode, and nonneural progenitor identity in vertebrates. PLoS Biol. 15, e2004045. http://dx.doi.org/10.1371/journal.pbio.2004045.

Plouhinec, J.-L., Roche, D.D., Pegoraro, C., Figueiredo, A.L., Maczkowiak, F., Brunet, L.J., Milet, C., Vert, J.-P., Pollet, N., Harland, R.M., Monsoro-Burq, A.H., 2014. Pax3 and Zic1 trigger the early neural crest gene regulatory network by the direct activation of multiple key neural crest specifiers. Dev. Biol. 386, 461–472. http://dx.doi.org/10.1016/j.ydbio.2013.12.010.

Pramanik, K.C., Kudugunti, S.K., Fofaria, N.M., Moridani, M.Y., Srivastava, S.K., 2013. Caffeic acid phenethyl ester suppresses melanoma tumor growth by inhibiting PI3K/AKT/XIAP pathway. Carcinogenesis 34, 2061–2070. http://dx.doi.org/10.1093/carcin/bgt154.

Rabadán, M.A., Usieto, S., Lavarino, C., Martí, E., 2013. Identification of a putative transcriptome signature common to neuroblastoma and neural crest cells. Dev. Neurobiol. 73, 815–827. http://dx.doi.org/10.1002/dneu.22099.

Rinon, A., Molchadsky, A., Nathan, E., Yovel, G., Rotter, V., Sarig, R., Tzahor, E., 2011. P53 coordinates cranial neural crest cell growth and epithelial-mesenchymal transition/delamination processes. Development 138, 1827–1838. http://dx.doi.org/10.1242/dev.053645.

Risso, G., Blaustein, M., Pozzi, B., Mammi, P., Srebrow, A., 2015. Akt/PKB: one kinase, many modifications. Biochem. J. 468, 203–214. http://dx.doi.org/10.1042/BJ20150041.

Roger, L., Gadea, G., Roux, P., 2006. Control of cell migration: a tumour suppressor function for p53? Biol. Cell 98, 141–152. http://dx.doi.org/10.1042/BC20050058.

Ruan, G.X., Kazlauskas, A., 2011. Focus on molecules: Akt (PKB). Exp. Eye Res. 93, 570–571. http://dx.doi.org/10.1016/j.exer.2010.06.016.

Rubinstein, A.L., Lee, D., Luo, R., Henion, P.D., Halpern, M.E., 2000. Genes dependent on zebrafish cyclops function identified by AFLP differential gene expression screen. Genesis 26, 86–97. http://dx.doi.org/10.1002/(SICI)1526-968X(200001)26:1<86::AID-GENE11>3.0.CO;2-Q.

Saadi, I., Alkuraya, F.S., Gisselbrecht, S.S., Goessling, W., Cavallesco, R., Turbe-Doan, A., Petrin, A.L., Harris, J., Siddiqui, U., Grix, A.W., Hove, H.D., Leboulch, P., Glover, T.W., Morton, C.C., Richieri-Costa, A., Murray, J.C., Erickson, R.P., Maas, R.L., Maas, R.L., 2011. Deficiency of the cytoskeletal protein SPECC1L leads to oblique facial clefting. Am. J. Hum. Genet. 89, 44–55. http://dx.doi.org/10.1016/j.ajhg.2011.05.023.

Santoro, M., Wong, W.T., Aroca, P., Santos, E., Matoskova, B., Grieco, M., Fusco, A., Paolo, P., Fiore2, D., 1994. An epidermal growth factor receptor/ret chimera generates mitogenic and transforming signals: evidence for a ret-specific signaling pathway. Mol. Cell. Biol. 14, 663–675.

Sarbassov, D.D., Guertin, D.A., Ali, S.M., Sabatini, D.M., 2005. Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. (80-.)Science 307, 1098–1101. http://dx.doi.org/10.1126/science.1106148.

Sasai, Y., 2005. Developmental biology: a blank canvas no more. Nature. http://dx.doi.org/10.1038/435433a.

Sauka-Spengler, T., Bronner-Fraser, M., 2008. A gene regulatory network orchestrates neural crest formation. Nat. Rev. Mol. Cell Biol. 9, 557–568. http://dx.doi.org/10.1038/nrm2428.

Schuchardt, A., D'Agati, V., Larsson-Blomberg, L., Costantini, F., Pachnis, V., 1994. Defects in the kidney and enteric nervous system of mice lacking the tyrosine kinase receptor Ret. Nature 367, 380–383. http://dx.doi.org/10.1038/367380a0.

Schuff, M., Siegel, D., Bardine, N., Oswald, F., Donow, C., Knöchel, W., 2010. FoxO genes are dispensable during gastrulation but required for late embryogenesis in Xenopus laevis. Dev. Biol. 337, 259–273. http://dx.doi.org/10.1016/j.ydbio.2009.10.036.

Shirakabe, K., Wakatsuki, S., Kurisaki, T., Fujisawa-Sehara, A., 2000. Roles of meltrin B/ADAM19 in the processing of neuregulin. J. Biol. Chem., 276. http://dx.doi.org/10.1074/jbc.M007913200.

Shukla, V., Coumoul, X., Wang, R.-H., Kim, H.-S., Deng, C.-X., 2007. RNA interference

and inhibition of MEK-ERK signaling prevent abnormal skeletal phenotypes in a mouse model of craniosynostosis. Nat. Genet. 39, 1145–1150. http://dx.doi.org/10.1038/ng2096.

Simões-Costa, M., Stone, M., Bronner, M.E., 2015. Axud1 integrates Wnt signaling and transcriptional inputs to drive neural crest formation. Dev. Cell 34, 544–554. http://dx.doi.org/10.1016/j.devcel.2015.06.024.

Soriano, P., 1997. The PDGFα receptor is required for neural crest cell development and for normal patterning of the somites. Development 124, 2691–2700.

Srinivasan, S., Anitha, M., Mwangi, S., Heuckeroth, R.O., 2005. Enteric neuroblasts require the phosphatidylinositol 3-kinase/Akt/Forkhead pathway for GDNF-stimulated survival. Mol. Cell. Neurosci. 29, 107–119. http://dx.doi.org/10.1016/j.mcn.2005.02.005.

Steventon, B., Araya, C., Linker, C., Kuriyama, S., Mayor, R., 2009. Differential requirements of BMP and Wnt signalling during gastrulation and neurulation define two steps in neural crest induction. Development 136, 771–779. http://dx.doi.org/10.1242/dev.029017.

Stokoe, D., Stephens, L.R., Copeland, T., Gaffney, P.R., Reese, C.B., Painter, G.F., Holmes, A.B., McCormick, F., Hawkins, P.T., 1997. Dual role of phosphatidylinositol-3,4,5-trisphosphate in the activation of protein kinase B. Science 277, 567–570.

Streit, A., 2002. Extensive cell movements accompany formation of the otic placode. Dev. Biol. 249, 237–254. http://dx.doi.org/10.1006/dbio.2002.0739.

Su, N., Jin, M., Chen, L., 2014. Role of FGF/FGFR signaling in skeletal development and homeostasis: learning from mouse models. Bone Res. 2, 14003. http://dx.doi.org/10.1038/boneres.2014.3.

Tallquist, M.D., Soriano, P., 2003. Cell autonomous requirement for PDGFRalpha in populations of cranial and cardiac neural crest cells. Development 130, 507–518. http://dx.doi.org/10.1242/dev.00241.

Taylor, S.R., Markesbery, M.G., Harding, P.A., 2014. Heparin-binding epidermal growth factor-like growth factor (HB-EGF) and proteolytic processing by a disintegrin and metalloproteinases (ADAM): a regulator of several pathways. Semin. Cell Dev. Biol. 28, 22–30. http://dx.doi.org/10.1016/j.semcdb.2014.03.004.

Theveneau, E., Mayor, R., 2014. Neural crest cell migration. In: Paul Trainor (Ed.), Neural Crest Cells. Elsevier, Academic Press (Elsevier), 73–88. http://dx.doi.org/10.1016/B978-0-12-401730-6.00004-1, ISBN 978-0-12-401730-6.

Tokino, T., Nakamura, Y., 2000. The role of p53-target genes in human cancer. Crit. Rev. Oncol. Hematol. 33, 1–6. http://dx.doi.org/10.1016/S1040-8428(99)00051-7.

Tribulo, C., 2003. Regulation of Msx genes by a Bmp gradient is essential for neural crest specification. Development 130, 6441–6452. http://dx.doi.org/10.1242/dev.00878.

van Weering, D.H., Bos, J.L., 1997. Glial cell line-derived neurotrophic factor induces Ret-mediated lamellipodia formation. J. Biol. Chem. 272, 249–254. http://dx.doi.org/10.1074/JBC.272.1.249.

Vastag, L., Jorgensen, P., Peshkin, L., Wei, R., Rabinowitz, J.D., Kirschner, M.W., 2011. Remodeling of the metabolome during early frog development. PLoS One, 6. http://dx.doi.org/10.1371/journal.pone.0016881.

Vasudevan, H.N., Mazot, P., He, F., Soriano, P., 2015. Receptor tyrosine kinases modulate distinct transcriptional programs by differential usage of intracellular pathways. Elife 4, 1–22. http://dx.doi.org/10.7554/eLife.07186.

Visciano, C., Liotti, F., Prevete, N., Cali', G., Franco, R., Collina, F., De Paulis, A., Marone, G., Santoro, M., Melillo, R.M., 2015. Mast cells induce epithelial-to-mesenchymal transition and stem cell features in human thyroid cancer cells through an IL-8-Akt-slug pathway. Oncogene 34, 5175–5186. http://dx.doi.org/10.1038/onc.2014.441.

Wang, J., Xiao, Y., Hsu, C.-W., Martinez-Traverso, I.M., Zhang, M., Bai, Y., Ishii, M., Maxson, R.E., Olson, E.N., Dickinson, M.E., Wythe, J.D., Martin, J.F., 2016. Yap and Taz play a crucial role in neural crest-derived craniofacial development. Development 143, 504–515. http://dx.doi.org/10.1242/dev.126920.

Wang, T., Stromberg, G.P., Whitney, J.T., Brower, N.W., Klymkowsky, M.W., Parent, J.M., 2006. Sox3 expression identifies neural progenitors in persistent neonatal and adult mouse forebrain germinative zones. J. Comp. Neurol. 502, 275–290. http://dx.doi.org/10.1002/cne.

Wilson, N.R., Olm-Shipman, A.J., Acevedo, D.S., Palaniyandi, K., Hall, E.G., Kosa, E., Stumpff, K.M., Smith, G.J., Pitstick, L., Liao, E.C., Bjork, B.C., Czirok, A., Saadi, I., 2016. SPECC1L deficiency results in increased adherens junction stability and reduced cranial neural crest cell delamination. Nat. Publ. Gr., 1–15. http://dx.doi.org/10.1038/srep17735.

Xia, Z., Tong, X., Liang, F., Zhang, Y., Kuok, C., Zhang, Y., Liu, X., Zhu, Z., Lin, S., Zhang, B., 2013. Eif3ba regulates cranial neural crest development by modulating p53 in zebrafish. Dev. Biol. 381, 83–96. http://dx.doi.org/10.1016/j.ydbio.2013.06.009.

Yang, P.H., Cheung, W.K.C., Peng, Y., He, M.L., Wu, G.Q., Xie, D., Jiang, B.H., Huang, Q.H., Chen, Z., Lin, M.C.M., Kung, H.F., 2008. Makorin-2 is a neurogenesis inhibitor downstream of phosphatidylinositol 3-kinase/Akt (PI3K/Akt) signal. J. Biol. Chem. 283, 8486–8495. http://dx.doi.org/10.1074/jbc.M704768200.

Yang, T., Moore, M., He, F., 2017. Pten regulates neural crest proliferation and differentiation during mouse craniofacial development. Dev. Dyn.. http://dx.doi.org/10.1002/dvdy.

Zhang, X., Wang, Q., Ling, M.-T., Wong, Y.C., Leung, S.C.L., Wang, X., 2007. Anti-apoptotic role of TWIST and its association with Akt pathway in mediating taxol resistance in nasopharyngeal carcinoma cells. Int. J. Cancer 120, 1891–1898. http://dx.doi.org/10.1002/ijc.22489.