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Sonic Hedgehog promotes the survival of neural crest cells by limiting apoptosis induced by the dependence receptor CDON during branchial arch development

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ABSTRACT

Cell-adhesion molecule-related/Downregulated by Oncogenes (CDO or CDON) was identified as a receptor for the classic morphogen Sonic Hedgehog (SHH). It has been shown that, in cell culture, CDO also behaves as a SHH dependence receptor: CDO actively triggers apoptosis in absence of SHH via a proteolytic cleavage in CDO intracellular domain. We present evidence that CDO is also pro-apoptotic in the developing neural tube where SHH is known to act as a survival factor. SHH, produced by the ventral foregut endoderm, was shown to promote survival of facial neural crest cells (NCCs) that colonize the first branchial arch (BA1). We show here that the survival activity of SHH on neural crest cells is due to SHH-mediated inhibition of CDO pro-apoptotic activity. Silencing of CDO rescued NCCs from apoptosis observed upon SHH inhibition in the ventral foregut endoderm. Thus, the pair SHH/dependence receptor CDO may play an important role in neural crest cell survival during the formation of the first branchial arch.

1. Introduction

Sonic Hedgehog (SHH) is a multifunctional cue with multiple known functions during both embryonic development and through adulthood. SHH is a classic morphogen known to regulate many developmental processes including ventrodorsal patterning of the neural tube, establishment of limb polarity and development of the foregut and axio-cranial skeleton [1,2]. However, SHH is not only implicated in the promotion of differentiation but was also more recently described as a survival factor. Indeed, inhibition of SHH in the developing neural tube induces massive neuroepithelial cell death [3,4]. Similarly, SHH produced by the ventral foregut endoderm promotes survival of facial neural crest cells (NCCs) that colonize the first branchial arch (BA1), induces the patterning of BA1 [5] and is therefore an early signal for jaw development during facial skeleton formation.

SHH canonical signaling includes its interaction with the twelve-transmembrane receptor Patched 1 (Ptc). Binding of SHH to Ptc prevents Ptc suppressive effect on Smoothened (Smo), an orphan seven-transmembrane receptor that initiates a signaling pathway leading to the activation of the glioma-associated (Gli) family of transcription factors. However, SHH also appears to interact with other single-transmembrane proteins such as Cell-adhesion molecule-related/Downregulated by Oncogenes (CDO) and Brother of CDO (BOC), two homologous members of the Neural Cell Adhesion Molecule (N-CAM) family [6–9]. CDO extracellular domain was indeed shown to directly bind SHH in a calcium-dependent and heparin-independent manner in mammals. This interaction enhances SHH–Ptc–Smo signaling in specific subregions of SHH expression [10].

CDO resembles in many aspects to DCC (Deleted in Colorectal Carcinoma), the prototypical netrin-1 dependence receptor [11]. Dependence receptors share the property of creating cellular state of dependence upon their ligand by inducing apoptosis when unbound by their ligand [12]. We have shown that CDO is a dependence receptor: upon forced expression, CDO triggers apoptosis in various cancer cell lines, a pro-apoptotic activity blocked by the addition of SHH in the milieu [13]. CDO pro-apoptotic activity requires the caspase cleavage of CDO in its intracellular domain leading to the exposure of a pro-apoptotic domain able to recruit and activate the apical caspase-9. We proposed that this pro-apoptotic activity is a safeguard mechanism to limit cancer

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progression [13]. Here, we investigated the *in vivo* relevance of the dependence receptor activity of CDO during embryonic development and more specifically during branchial arch formation.

2. Materials and methods

2.1. Cell lines, transfection procedures, cell death assays, reagents

Human embryonic kidney HEK293T and NIH3T3 cell lines were cultured in DMEM medium (Gibco®, Invitrogen) containing 10% fetal bovine serum. Recombinant human SHH was purchased from R&D system (Minneapolis, MN). 5E1 hybridoma cells producing a SHH-blocking antibody (Developmental Studies Hybridoma Bank) and IgG1 hybridoma cells producing an isotypic unrelated mouse antibody were maintained in HybriCare medium (ATCC). Cell death assays were performed as in [13].

2.2. Plasmid constructs

Mouse CDO fragments were PCR amplified using pBABE-mCDO-Myc as described before [13,14]. A Flag tag was added to each construct. Mouse CDO IC fragments encompass sequences coding for CDO residues Leu^{984} to Thr^{1250}. Point mutations Asp (GAT or GAC) to Asn (AAT or AAC) were created using the QuickChange site directed mutagenesis strategy (Stratagene). The dominant negative mutant for CDO (CDO-DN) thus corresponds to CDO IC fragment with Asp^{1153} to Asn and Asp^{1164} to Asn point mutations. Patched1 construct and dominant negative mutant for Patched1 (pcDNA3.1-PTC1-DN-HA) have been previously described [4]. For *in ovo* chick electroporation, most constructs were based on pMiW vector which was also used as empty vector. List of all primers used is available upon request.

2.3. Chick in ovo experiments, immunofluoresence and TUNEL staining on chick embryos

Cell-death analysis in the developing chick neural tube was performed using fertilized chicken eggs obtained from a local farm and incubated at 38 °C. Embryos were staged according to Hamburger and Hamilton (HH). Chick embryos were electroporated with purified plasmid DNA (4.5 μg/μl) allowing expression in chicken (constructs based on pMIW vector).

For neural tube electroporation, chick embryos of stage HH10-11 were injected with plasmid DNA into the lumen of the neural tube and electroporated with specifically designed electrodes, only in one side of the neural tube. In each condition, GFP was co-electroporated to check the electroporation efficiency. Twenty-four hours after electroporation, live embryos were harvested, fixed overnight in 4% PFA, embedded in 7.5% gelatin – 0.12 M sucrose and 20 μm sections were performed. Detection and quantification of apoptosis by TUNEL was performed using the *In Situ* Cell Death Detection Kit (Roche). For each embryo, TUNEL positive cells of the electroporated and non-electroporated sides of the neural tube were counted in more than ten sections. The ratio of the number of TUNEL-positive cells from the electroporated side to that of the non-electroporated side was then calculated. On the same slides, immunofluorescence was performed to detect the expression of each electroporated construct, as described above. For each condition, results from at least six embryos were used to determine a mean ratio.

For *in ovo* experiments on NCCs migrating to BA1, chick embryos were bilaterally electroporated at 3ss- to 5-ss with pMIW empty vector, pMIW-CDO-DN-Flag or pMIW-PTC1-DN-HA. When embryos reached 5-ss to 7-ss stage, a glass needle was placed at the midbrain–hindbrain level, and 5E1 or IgG1 secreting hybridoma cells were pressure injected into the lateral head mesenchyme, as previously described [15]. Thirty six hours later, live embryos were harvested, fixed overnight in 4% PFA and subjected to whole mount immunofluorescent staining to detect antibodies secreted by injected hybridoma cells or TUNEL staining to detect apoptotic cells in the BA1.

Immunofluorescence was performed from slide incubated at room temperature for 2 h with a primary antibody recognizing the human CDO (1:200, R&D systems, Minneapolis, MN), the FlagM2 tag (1/400, Sigma), Ptc (1/200, Santa Cruz) or the HA tag (1/500, Sigma). After rinsing in phosphate buffer saline, the slides were incubated with an Alexa-488-Donkey or Cy3-Donkey anti-Goat antibody (Molecular Probes), or Cy5-Donkey anti-Mouse/anti-rabbit/anti-goat antibodies (Jackson ImmunoResearch, Suffolk, UK) (Molecular Probes) respectively. Nuclei were visualized with Hoechst staining. Fluorescence imaging was performed with AxioVision Release 4.6 software.

3. Results

3.1. CDO induces apoptosis in the developing neural tube

The dependence receptor activity of CDO was initially demonstrated using ectopic expression of CDO in various cancer cell lines [13]. To monitor whether CDO also triggers apoptosis in normal cells *in vivo*, we took advantage of the chick developing neural tube model. In the developing neural tube, SHH is produced by the notochord (NT) and the floor plate (FP), which induces a ventrodorsal gradient of SHH and promotes the differentiation of ventral neurons [16]. However, it was elegantly demonstrated that SHH acts in the developing neural tube not only as a morphogen but also as a survival factor for neuroepithelial cells [3,4]. We first forced the expression of CDO in one side of the neural tube of E1.5 chick embryos *in ovo* via electroporation (See schematic representation in Fig. 1A). As shown in Fig. 1B and C, the expression of CDO, detected by immunofluorescence in the electroporated side of the chick neural tube, was associated with an increase in the number of TUNEL-stained cells as compared to the non-electroporated side, but also as compared to neural tubes electroporated with a mock construct. In agreement with the SHH distribution along the neural tube, apoptosis was rather detected in the third dorsal part of the neural tube (see quantification in Fig. 1C). Thus forced expression of CDO in the neuroepithelium is associated with apoptosis induction.

3.2. Development of a dominant negative mutant for CDO pro-apoptotic activity

To search for the functional relevance of CDO pro-apoptotic activity *in vivo*, we first looked for a tool that would specifically inhibit CDO pro-apoptotic activity. Of interest, the intracellular domain of most dependence receptors mutated at its caspase cleavage site(s) specifically behaves as a dominant negative for the pro-apoptotic activity of the respective endogenous dependence receptor [4,17,18]. CDO was shown to be cleaved by caspase *in vitro* and we described one main cleavage site after aspartic acid residue Asp^{1153} and one cryptic site after Asp^{1164} ([13], See schematic representation Fig. 2A). We thus analyzed whether the intracellular domain of CDO mutated at the caspase cleavage site(s) (CDO-IC-D1153 or CDO-IC-D1153/1164N) antagonized CDO-induced apoptosis. We first analyzed cell death after ectopic expression of CDO in HEK293T cells. As expected for the dependence receptor paradigm, expression of CDO alone triggered apoptosis as measured by caspase-3 activity and by TUNEL staining (Figs. 2B and S1A). As shown in Fig. 2B, CDO-IC-D1153N markedly inhibited full-length CDO-induced HEK293T cell death. Furthermore, the CDO intracellular domain mutated on both caspase cleavage sites Asp^{1153} and Asp^{1164} (CDO-IC-D1153/1164N) completely inhibited full-length

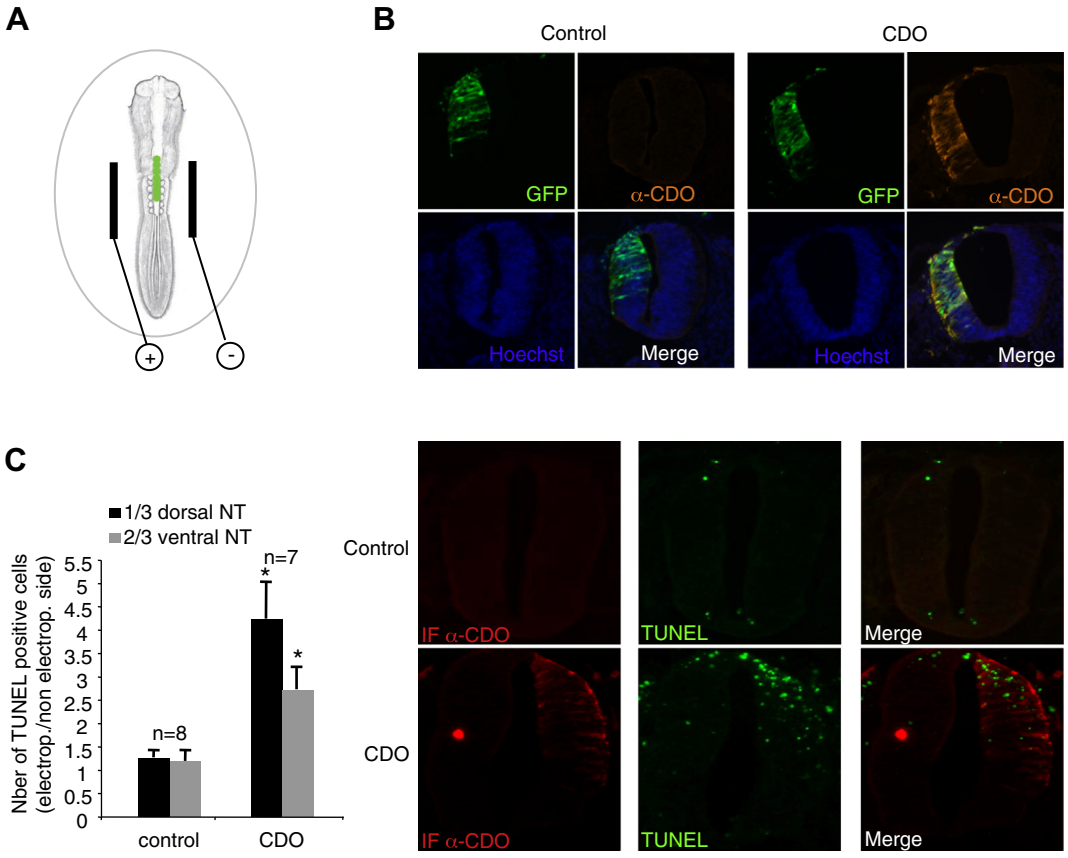

Fig. 1. CDO induces apoptosis in the developing neural tube. (A) Schematic representation of the *in ovo* electroporation method of HH-10 chick embryos. Electrodes are represented by + and – schemes. (B) CDO immunofluorescence staining of chick embryos neural tubes electroporated with mock or CDO encoding plasmids. CDO immunodetection is shown in orange. Electroporation efficiency is controlled with GFP green fluorescence. Hoechst staining is in blue. (C) Left panel: quantification of the number of TUNEL positive cells in the electroporated side versus the non-electroporated side for each condition shown in right panels. Quantification was performed separately in the dorsal third and in the ventral two-thirds of the neural tube. The number of embryos used for each condition is indicated above the graph. Error bars indicate s.e.m. Statistical treatment of the data was performed using a two-sided Mann–Whitney test compared to mock-transfected condition (*$P < 0.05$). Right panels: representative images of CDO immunofluorescence staining (in red) and TUNEL staining (in green) of chick embryos neural tubes electroporated with mock or CDO encoding plasmids are shown.

CDO-induced apoptotic cell death (Figs. 2B and S1A) while it had no effect on DCC or Ptc-induced apoptosis (Fig. 2C). We thus analyzed whether this mutant affected the gene transcription response triggered by the canonical Smo/Gli pathway by measuring its effect on the transactivation of a Gli-1-Luciferase reporter gene. As shown in Fig. S1B, CDO-IC-D1153/1164N failed to alter the gene transcription response induced by the Smo–Gli pathway. Thus, CDO-IC-D1153/1164N, named hereafter CDO-DN, acts as a specific dominant negative mutant for CDO full-length pro-apoptotic activity *in vitro*.

We then assessed whether CDO-DN acts as a dominant negative *in vivo*. To do so, CDO-DN alone or in combination with full-length CDO was overexpressed in E1.5 chick neural tube via unilateral electroporation. As shown in Fig. 2DE, while CDO overexpression induced an increased number of apoptotic cells specifically in the electroporated side of the chick neural tube as measured by TUNEL staining, this effect was completely prevented in presence of CDO-DN. CDO-DN was however unable to inhibit the death induced upon Ptc electroporation, while Ptc dominant negative mutant (Ptc-DN) [4] failed to inhibit the death induced upon CDO electroporation. Thus, CDO-DN specifically represses full-length CDO pro-apoptotic activity and so acts as a dominant negative *in vivo*.

3.3. The pair SHH/dependence receptor CDO controls the death of neural crest cells during the first branchial arch development

SHH has recently been implicated in the facial skeleton development and more precisely it was shown that SHH produced by the ventral foregut endoderm is crucial for the survival of facial neural crest cells (NCCs) that colonize the first branchial arch (BA1) [5,19]. In chick embryos, the experimental excision of the entire forehead before the onset of NCCs migration (HH-7/8 stage, seven somites) is associated with massive NCCs death in the first branchial arch, an effect blocked by addition of recombinant SHH. To assay whether CDO, which is known to be expressed in the BA1 during the early development [20], is implicated in the observed NCCs death in the BA1, we electroporated chick embryos with CDO-DN or mock constructs while interfering with SHH by injecting SHH-blocking antibody-secreting hybridoma cells (5E1) in the lateral mesenchyme before HH-7/8 stage [15] (experimental setting in Fig. 3A). The blocking antibody secreted by hybridoma cells was detected 24 h after injection in the lateral mesenchyme (Fig. 3B). As shown in Figs. 3C and S2, the SHH-blocking antibody, but not the non-related isotypic immunoglobulin (IgG1), induced a wave of cell death in the BA1 of control-electroporated embryos. In this setting, CDO-DN electroporation fully blocked cell death in the developing BA1 (Fig. 3C). Of interest, when a similar experiment was performed using Ptc-DN instead of CDO-DN, cell death was not inhibited (Fig. S2), thus suggesting that in this model, SHH acts as a survival factor by specifically blocking CDO-mediated apoptosis.

4. Discussion

Together with recent work [13], we propose here that CDO is not only part of a receptor complex allowing the adequate

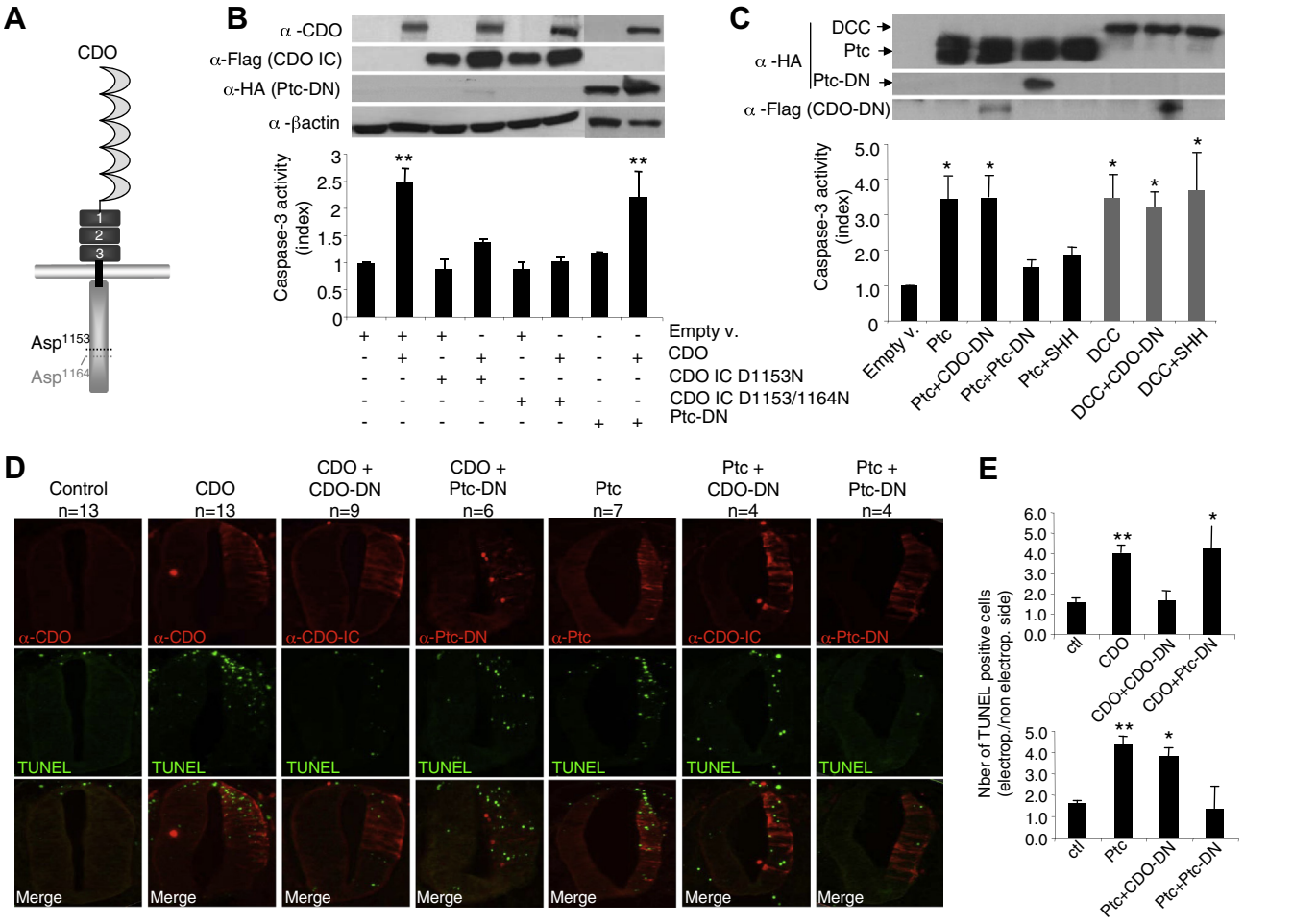

Fig. 2. CDO-DN behaves as a specific dominant negative for CDO pro-apoptotic activity *in vitro* and *in vivo*. (A) Schematic representation of CDO and its main (D1153) and secondary (D1164) caspase cleavage sites. (B) Apoptotic cell death induction as measured by caspase-3 activity was quantified in HEK293T cells transfected with full-length CDO alone or together with CDO-IC domain mutated on one (CDO-IC-D1153N) or two (CDO-IC-D1153/64N) caspase cleavage site(s), or Ptc dominant negative (Ptc-DN). Upper panel shows detection of CDO (α-CDO), CDO-IC mutants (α-Flag) and Ptc-DN (α-HA) proteins by immunoblot. CDO-IC-D1153/64N, named hereafter CDO-DN, behaves as a dominant negative for CDO pro-apoptotic activity. (C) Apoptotic cell death induction as measured by caspase-3 activity was quantified in HEK293T cells transfected with Ptc or DCC alone or together with CDO-DN or Ptc-DN constructs. Upper panel shows detection of Ptc, DCC and Ptc-DN (α-HA), and CDO-DN (α-Flag). For (B) and (C), error bars indicate s.e.m. Data are means of at least three independent assays. Statistical treatment of the data was performed using a two-sided Mann–Whitney test compared to mock-transfected condition ($^{*}P < 0.05$; $^{**}P < 0.01$). (D) Immunofluorescence (upper panels) and TUNEL staining (median panels) on chick embryos neural tubes electroporated with mock, CDO or Ptc alone or together with CDO-DN or Ptc-DN encoding plasmids. Immunodetection of full length CDO (anti-CDO antibody), CDO-IC domain (detection of both CDO and CDO-DN with anti-Flag antibody), Ptc (anti-Ptc antibody) and Ptc-DN (anti-HA antibody) is shown in red. TUNEL staining is in green. TUNEL and immunofluorescence staining are shown as merge in lower panels. The number of embryos used for each condition is indicated above the panels. (E) Quantification of the number of TUNEL positive cells in the electroporated side versus the non-electroporated side for each condition presented in (C). Error bars indicate s.e.m. Statistical treatment of the data was performed using a two-sided Mann–Whitney test compared to mock-transfected condition ($^{**}P < 0.01$; $^{*}P < 0.05$).

canonical signaling occurring upon SHH binding to Ptc. Independently of Ptc and of the canonical signaling Ptc/Smo/Gli, CDO actively triggers apoptosis, a mechanism occurring *in vivo* to adequately control neural crest cells migration during formation of the first branchial arch. SHH and its dependence receptor CDO are thus playing a regulatory role to control the number of NCCs. As hypothesized by the dependence receptor paradigm [21], this may represent an important mechanism to allow only a limited number of NCCs in the BA1 relatively to a limited amount of cues needed to define a precise migration pathway.

An intriguing consequence of this work is the fact that among the known SHH receptors – i.e., Ptc, HHIP1, GAS1, BOC and CDO – at least two of them, Ptc and CDO, behave as dependence receptors. Thus, SHH mediates cell survival by blocking both CDO and Ptc dependence receptors pro-apoptotic activity, two receptors that are often expressed in the same cells and have been shown to work together to mediate the classic SHH/Gli signaling [6,7]. Why would a cell need more than one SHH receptor to undergo apoptosis upon SHH unavailability? This could suggest either redundancy in a common mechanism of cell death induction upon limitation in SHH availability to ensure apoptosis of an unwanted/inadequately located cell or conversely a double-check mechanism that secures only the death of unwanted cells. In the developing neural tube, both CDO and Ptc are expressed in neuroepithelial cells and we have shown that both Ptc and CDO are able to trigger neuroepithelial cell death [4,22] and thus SHH-mediated survival occurs probably by blocking both Ptc and CDO pro-apoptotic activity. In the specific case of migrating NCCs that colonize the first branchial arch, Ptc expression is barely detected and the absence of cell death rescue by the Ptc dominant negative mutant upon SHH inhibition further supports the view that SHH survival activity occurs mainly through inhibition of CDO pro-apoptotic activity. Moreover, in vertebrates, BOC, CDO and GAS1 were recently presented as obligate co-receptors of Ptc in different developmental processes including neural tube and limb patterning, and maintenance of motor neurons [9,23]. It will then be interesting to study

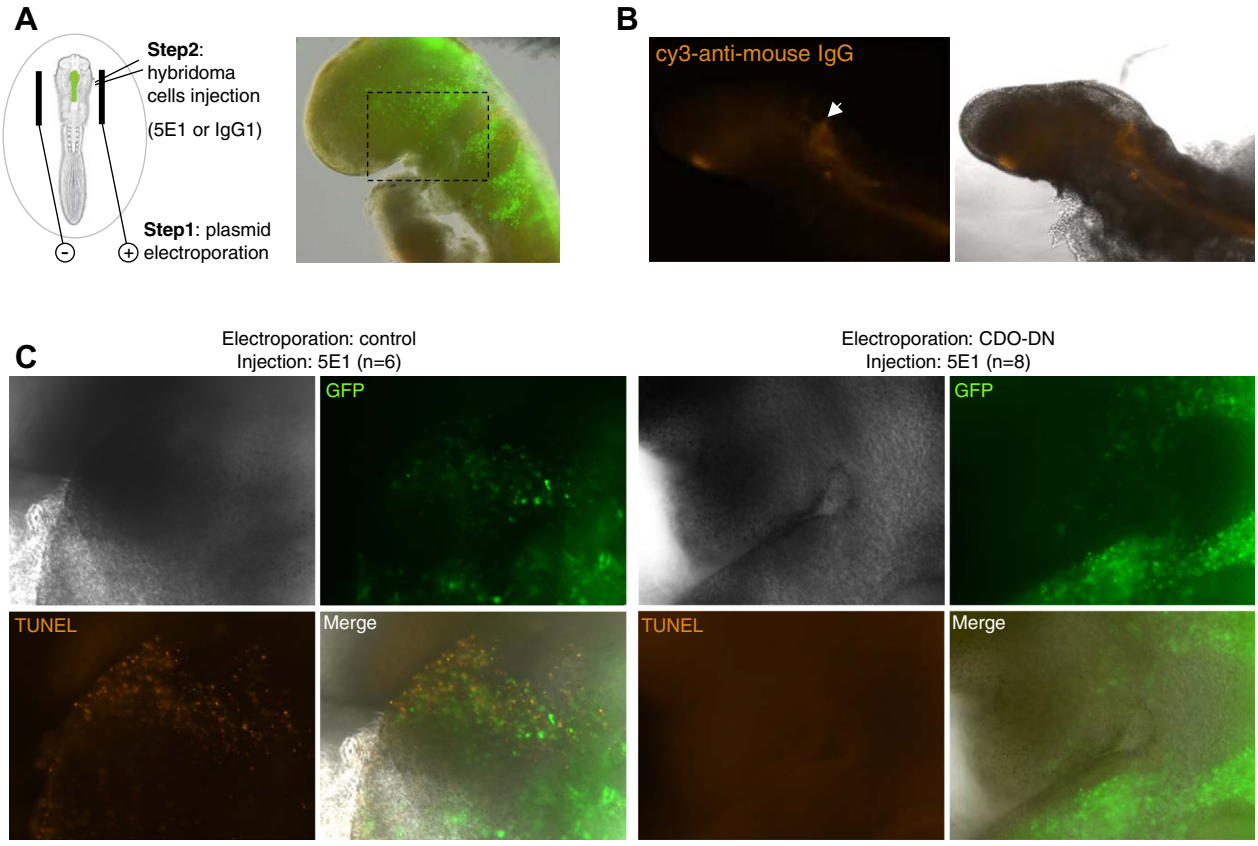

Fig. 3. The pair SHH/dependence receptor CDO controls neural crest cells death during first Branchial Arch development. (A) Left panel: schematic representation of the *in ovo* electroporation method of HH-7/8 chick embryos, followed by injection of antibody-secreting hybridoma cells in the lateral mesenchyme. Right panel: representative image of a chick embryo electroporated with a GFP-construct (in green), injected with control antibody-secreting hybridoma cells and then subjected to TUNEL staining (in orange). The dotted area highlights the zone observed at higher magnification in (C). (B) Immunodetection of hybridoma cells injected in the lateral mesenchyme of chick embryos 24 h after injection. The secretion of mouse immunoglobulins by 5E1- or IgG1-secreting hybridoma cells was evidenced with a cy3-anti-mouse antibody (white arrowhead). (C) *In toto* TUNEL staining performed on chick embryos 36 h after electroporation of mock or CDO-DN encoding constructs and injection of SHH-blocking antibody (5E1) secreting hybridoma cells. Electroporation efficiency is controlled with GFP green fluorescence. TUNEL staining is shown in orange. Arrows point at the first branchial arch in formation. The number of embryos analyzed is indicated on each illustrated condition.

the potential impact of BOC and/or GAS1 on CDO-induced cell death, depending on SHH availability and on the cellular/tissue context. More generally, further work will be necessary to analyze whether CDO pro-apoptotic activity is also important in other aspect of SHH pleiotropic implications during development.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.08.134.

References

[1] T.M. Jessell, Neuronal specification in the spinal cord: inductive signals and transcriptional codes, Nat. Rev. Genet. 1 (2000) 20–29.
[2] P.W. Ingham, A.P. McMahon, Hedgehog signaling in animal development: paradigms and principles, Genes Dev. 15 (2001) 3059–3087.
[3] J.B. Charrier, F. Lapointe, N.M. Le Douarin, M.A. Teillet, Anti-apoptotic role of Sonic Hedgehog protein at the early stages of nervous system organogenesis, Development 128 (2001) 4011–4020.
[4] C. Thibert, M.A. Teillet, F. Lapointe, L. Mazelin, N.M. Le Douarin, P. Mehlen, Inhibition of neuroepithelial patched-induced apoptosis by sonic hedgehog, Science 301 (2003) 843–846.
[5] J.M. Brito, M.A. Teillet, N.M. Le Douarin, An early role for sonic hedgehog from foregut endoderm in jaw development: ensuring neural crest cell survival, Proc. Natl. Acad. Sci. USA 103 (2006) 11607–11612.
[6] T. Tenzen, B.L. Allen, F. Cole, J.S. Kang, R.S. Krauss, A.P. McMahon, The cell surface membrane proteins Cdo and Boc are components and targets of the Hedgehog signaling pathway and feedback network in mice, Dev. Cell 10 (2006) 647–656.
[7] W. Zhang, J.S. Kang, F. Cole, M.J. Yi, R.S. Krauss, Cdo functions at multiple points in the Sonic Hedgehog pathway, and Cdo-deficient mice accurately model human holoprosencephaly, Dev. Cell 10 (2006) 657–665.
[8] D.C. Martinelli, C.M. Fan, Gas1 extends the range of Hedgehog action by facilitating its signaling, Genes Dev. 21 (2007) 1231–1243.
[9] B.L. Allen, J.Y. Song, L. Izzi, I.W. Althaus, J.S. Kang, F. Charron, R.S. Krauss, A.P. McMahon, Overlapping roles and collective requirement for the coreceptors GAS1, CDO, and BOC in SHH pathway function, Dev. Cell 20 (2011) 775–787.
[10] J.S. McLellan, X. Zheng, G. Hauk, R. Ghirlando, P.A. Beachy, D.J. Leahy, The mode of Hedgehog binding to Ihog homologues is not conserved across different phyla, Nature 455 (2008) 979–983.
[11] P. Mehlen, S. Rabizadeh, S.J. Snipas, N. Assa-Munt, G.S. Salvesen, D.E. Bredesen, The DCC gene product induces apoptosis by a mechanism requiring receptor proteolysis, Nature 395 (1998) 801–804.
[12] D. Goldschneider, P. Mehlen, Dependence receptors: a new paradigm in cell signaling and cancer therapy, Oncogene 29 (2010) 1865–1882.
[13] C. Delloye-Bourgeois, B. Gibert, N. Rama, J.G. Delcros, N. Gadot, J.Y. Scoazec, R. Krauss, A. Bernet, P. Mehlen, Sonic Hedgehog promotes tumor cell survival by inhibiting CDON pro-apoptotic activity, PLoS Biol. 11 (2013) e1001623.
[14] J.S. Kang, P.J. Mulieri, C. Miller, D.A. Sassoon, R.S. Krauss, CDO, a robo-related cell surface protein that mediates myogenic differentiation, J. Cell Biol. 143 (1998) 403–413.
[15] S.C. Ahlgren, M. Bronner-Fraser, Inhibition of sonic hedgehog signaling in vivo results in craniofacial neural crest cell death, Curr. Biol. 9 (1999) 1304–1314.

[16] N.M. Le Douarin, M.E. Halpern, Discussion point. Origin and specification of the neural tube floor plate: insights from the chick and zebrafish, Curr. Opin. Neurobiol. 10 (2000) 23–30.
[17] S. Tauszig-Delamasure, L.Y. Yu, J.R. Cabrera, J. Bouzas-Rodriguez, C. Mermet-Bouvier, C. Guix, M.C. Bordeaux, U. Arumae, P. Mehlen, The TrkC receptor induces apoptosis when the dependence receptor notion meets the neurotrophin paradigm, Proc. Natl. Acad. Sci. USA 104 (2007) 13361–13366.
[18] C. Furne, N. Rama, V. Corset, A. Chedotal, P. Mehlen, Netrin-1 is a survival factor during commissural neuron navigation, Proc. Natl. Acad. Sci. USA 105 (2008) 14465–14470.
[19] J.M. Brito, M.A. Teillet, N.M. Le Douarin, Induction of mirror-image supernumerary jaws in chicken mandibular mesenchyme by Sonic Hedgehog-producing cells, Development 135 (2008) 2311–2319.
[20] P.J. Mulieri, A. Okada, D.A. Sassoon, S.K. McConnell, R.S. Krauss, Developmental expression pattern of the cdo gene, Dev. Dyn. 219 (2000) 40–49.
[21] P. Mehlen, C. Thibert, Dependence receptors: between life and death, Cell. Mol. Life Sci. 61 (2004) 1854–1866.
[22] F. Mille, C. Thibert, J. Fombonne, N. Rama, C. Guix, H. Hayashi, V. Corset, J.C. Reed, P. Mehlen, The patched dependence receptor triggers apoptosis through a DRAL–caspase-9 complex, Nat. Cell Biol. 11 (2009) 739–746.
[23] L. Izzi, M. Levesque, S. Morin, D. Laniel, B.C. Wilkes, F. Mille, R.S. Krauss, A.P. McMahon, B.L. Allen, F. Charron, Boc and gas1 each form distinct shh receptor complexes with ptch1 and are required for shh-mediated cell proliferation, Dev. Cell 20 (2011) 788–801.