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Molecules in focus

Noggin

Carola Krause[1], Asja Guzman[2], Petra Knaus*

Institute for Chemistry and Biochemistry, Freie Universität Berlin, Thielallee 63, 14195 Berlin, Germany

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ABSTRACT

Metabologens initiate, promote and maintain morphogenesis and adult tissue homeostasis. Bone morphogenetic proteins (BMPs) which belong to the transforming growth factor-β (TGF-β) superfamily, represent a major class of metabologens that regulate ectoderm, mesoderm and endoderm derived tissue formation. In order to temporally and spatially control BMP initiated signaling cascades, a tight regulatory network is needed to maintain concinnity. There are a number of ways how BMP signaling can be inhibited or more likely be modified, among which the direct extracellular inhibition through cysteine-knot containing proteins from the DAN-, the twisted gastrulation-, chordin- and noggin-family is a classic. This review focuses on noggin and its impact on the vast array of BMP driven actions and thereby invites the ever-growing BMP research field to (re-) investigate noggin's function in detail.

1. Introduction

Noggin, encoded by the *NOG* gene, is a secreted homodimeric glycoprotein with a molecular mass of 64 kDa. Noggin was discovered by its ability to induce secondary axis formation in Xenopus embryos (Smith and Harland, 1992). Noggin rescues dorsal development in UV-induced ventralized Xenopus embryos and injection of the putative cDNA results in excessive head development, hence the name noggin (Smith and Harland, 1992). Nowadays, noggin is known to regulate a major class of metabologens, so-called bone morphogenetic proteins (BMPs). It is suggested that due to excessive BMP action noggin null mice display serious developmental abnormalities (McMahon et al., 1998; Tylzanowski et al., 2006).

2. Structure

Noggin's primary structure consists of an acidic amino-terminal and a cysteine-rich carboxy-terminal region. Through the formation of cystine knots, the carboxy-terminal region is used to classify BMP antagonists into three distinct subfamilies: CAN (eight-membered ring), twisted gastrulation (nine-membered ring) as well as chordin and noggin (ten-membered ring) (Avsian-Kretchmer and Hsueh, 2004).

The topology of noggin resembles BMPs in a two-fold axis of symmetry. The BMP-dimer is shaped like a butterfly with wings extending from a core body that mediates dimerization. Noggin dimerizes via a core body, from which 2 pairs of β-strands extend preceding by a N-terminal segment of about 20 amino acids, the so-called ĭclipĭ segment. This clip snakes around the BMP ligand and occludes the surfaces of the growth factor from binding to both the BMP type I and the type II receptors (Fig. 1) (Groppe et al., 2002). This resembles a conserved mode of binding as this clip is also described for crossveinless-2, another member of the antagonist superfamily (Zhang et al., 2008).

Thus far four BMP type I receptors (activin receptor-like kinase 1 (ALK1), Alk2, Alk3, and Alk6) and three BMP type II receptors (BMPR-II, activin type II receptor ActR-II and ActR-IIB) were found to be capable of specifically binding to certain BMPs (Table 1) (Miyazono et al., 2010). Binding of noggin to some of the BMPs inhibits those from binding and therefore activation of BMP receptors, thus blocking Smad-dependent and non-Smad signaling (Groppe et al., 2002).

In vitro experiments have shown that noggin binds with varying affinities to BMP-2, -4, -5, -6, -7, -13, -14 (Zimmerman et al., 1996; Song et al., 2010; Seemann et al., 2009). Thereby it inhibits BMP-2, -4, -5, -7, -13 and -14 mediated action, leaving BMP-3, -6, -9, -10 and -15 signaling unaffected (Table 1) (Gamer et al., 2005; Song et al., 2010; Seemann et al., 2009). Whether BMP binding and inhibition by noggin does always follow the described BMP7/noggin structural mode is up to date uncertain. Interestingly, binding of noggin to BMP-6 does not coincide with diminished BMP-6 activity in osteoblast differentiation *in vitro* (Song et al., 2010).

3. Expression, activation and turnover

Noggin is a pleiotropic factor, which is expressed both early in development as well as in later stages. During early gas-

* Corresponding author. Tel.: +49 3083852935; fax: +49 3083852936.
E-mail addresses: carola.krause@fu-berlin.de (C. Krause), asja_guzman@yahoo.com (A. Guzman), knaus@chemie.fu-berlin.de (P. Knaus).
[1] Tel.: +49 3083851187; fax: +49 3083852936.
[2] Tel.: +49 3083854779; fax: +49 3083852936.

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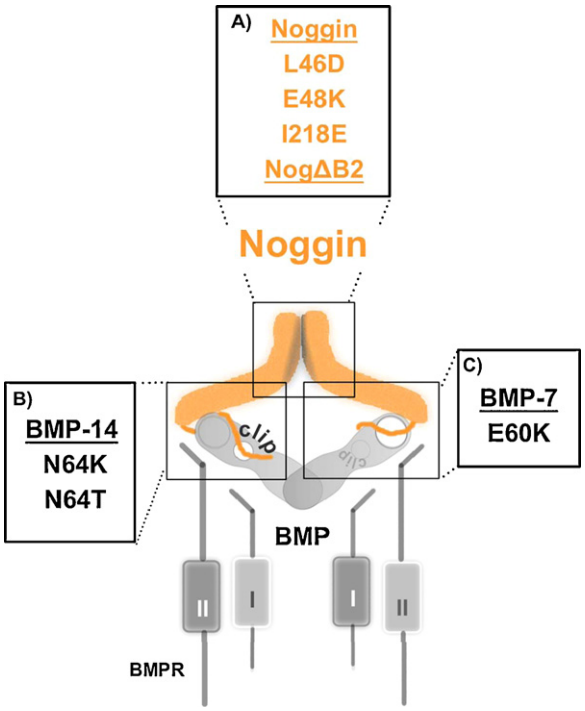

Fig. 1. Schematic representation of the suggested noggin-BMP-7 complex with indicated binding sites of BMP receptors (big circle = BMPRII; small circle = BMPRI). Noggin's core body (orange) embraces the BMP-7 dimer (grey) allowing its amino-terminal extensions (orange) to snake around it and prevent the surfaces of the growth factor from binding to both BMP type I and type II receptors. Indicated is the suggested ĭclip segment that creates high affinity BMP binding and shielding of the BMP type I receptor interface. Highlighted are noggin point mutations and deletion of the heparin binding site (ΔB2) in noggin (A) which possess lower BMP-7 binding capacity, and point mutations in BMP-14 (B) and BMP-7 (C), which are crucial for diminished susceptibility to noggin inhibition.

trulation noggin is produced by the Spemann organizer and antagonizes the action of BMP-2, -4, -7, leading to a BMP gradient directed dorsal–ventral patterning with subsequent germ layer formation (Fig. 2) (Smith and Harland, 1992; Zimmerman et al., 1996).

3.1. Noggin expression in ectoderm derivatives

The presence of noggin is essential in developmental structures derived from ectoderm such as the neural tube, tooth, hair follicle and eye development (McMahon et al., 1998). Although neural tube induction occurs in the absence of noggin, it was shown to be crucial for neurogenesis (McMahon et al., 1998; Lim et al., 2000). Noggin is expressed in the notochord and is augmented upon noradrenalin exposure in ectodermal derivatives (McMahon et al., 1998; Messenger et al., 1999). Thereupon, overexpression of noggin counteracts BMP-4 activity on neural precursor cells causing overproliferation of neural tissue (Fig. 2) (Bonaguidi et al., 2008). Noggin is also expressed in the dermal papilla and connective tissue of the hair follicle where it neutralizes BMP-4 hair follicle induction in embryonic skin organ cultures (Fig. 2) (Botchkarev et al., 1999). Interestingly, ectopic application of noggin and subsequent BMP-4 inhibition led to changes in tooth phenotype and to the development of molars instead of incisors (Tucker et al., 1998). Moreover, a crucial role of noggin has been implicated in eye development. Keratin 5 promoter-driven overexpression of noggin in the epidermis led to reduced apoptosis and retardation of cell differentiation in the eyelid epithelium (Sharov et al., 2003). Noggin is furthermore detected in the lens, retina and periocular mesenchyme and rescues ablated epiblast cell induced eye defects (Gerhart et al., 2009).

3.2. Noggin expression in mesoderm derivatives

Noggin is modestly expressed in mesoderm derived tissues and is required for embryonic somite and skeletal patterning (Fig. 2)

Table 1
Overview of members of the bone morphogenetic protein (BMP) family, their known BMP types I and BMP type II receptor and the detected tissue expression patterns. Whether the respective BMP is known to induce noggin expression is indicated with an arrow (↑) and its susceptibility to noggin inhibition is depicted via YES (inhibition through noggin detected) or NO (inhibition through noggin not detected). Activin receptor-like kinase (ALK); BMP type II receptor (BMPR-II); activin type II receptor (ActRII); transforming growth factor receptor III (TGFβ-RIII); central nervous system (CNS); unknown (–).

Ligand	Synonym	Receptors	BMP tissue location	Induction of noggin expression	Inhibition by noggin
BMP-2	BMP-2A	ALK-3,-6; BMPR-II; Endoglin; TGFP-RIII; Act-RIIA, -RIIB	Intramembranous bone, blood vessels, muscle, cartilage, teeth, liver, heart, sperm, hair follicle	↑	Yes
BMP-3	Osteogenin	Alk-4; Act-RIIB	Lung, kidney, intramembranous bone, cartilage, lung, teeth	–	No
BMP-4	BMP-2B	ALK-3, -6; Act-RIIA; BMPR-II; TGFP-RIII	Intramembranous bone, muscle, uterus, cartilage, teeth, kidney, gut, salivary gland, liver, pancreas, lung, heart, ovaries, sperm, hair follicle	↑	Yes
BMP-5	–	–	Intramembranous bone, cartilage, kidney, ureter, pancreas, lung, heart	–	Yes
BMP-6	Vgr-1	ALK-2, -3, -6;Act-RIIA,-RIIB; BMPR-II	Heart, cartilage, ureter, pancreas, heart, ovaries, epidermis, liver	↑	No
BMP-7	Op-1	Alk-3, -6; Act-RIIA,-RIIB; BMPR-II	Kidney, intramembranous bone, cartilage, synovium, eye, salivary gland, liver, pancreas, lung, heart, ovaries, sperm, epidermis	↑	Yes
BMP-8	Op-2, BMP8b	–	Intramembranous bone, ovaries, sperm	–	–
BMP-9	GDF-2	ALK-1, -2; Act-RIIA,-RIIB; BMPR-II; Endoglin	Liver, CNS	↑	No
BMP-10	–	ALK-1,-3, -6; Act-RIIA,-RIIB; BMPR-II	Heart	–	No
BMP-11	GDF-11	Alk-4, -5, -7; Act-RIIA,-RIIB	CNS	–	No
BMP-12	GDF-7, CDMP-3	ALK-3, -6; Act-RIIA; BMPR-II	Tendons, CNS, cartilage	–	–
BMP-13	GDF-6, CDMP-2	ALK-3, -6; BMPR-II	Tendons, cartilage	–	Yes
BMP-14	GDF-5, CDMP-1	Alk-6; Act-RIIA, -RIIB; BMPRII; TGFP-RIII	Cartilage, synovium, eye	↑	Yes
BMP-15	GDF-9B	ALK-6; Act-RIIA; BMPR-II	Ovaries	–	No

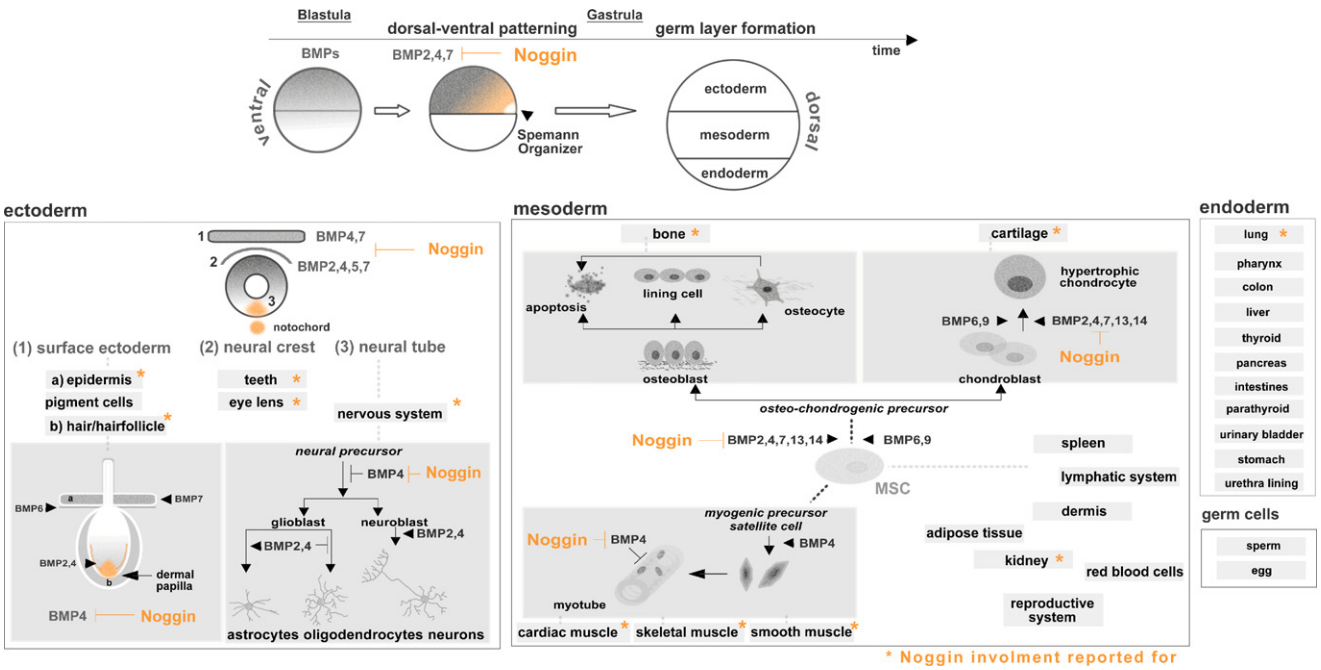

Fig. 2. Overview of noggin's function during gastrulation and tissue development. Highlighted is the antagonistic role of noggin (orange) on indicated BMP proteins during dorsal–ventral patterning of the gastrula and germ layer derived tissue derivates. Asterisks indicate tissues where noggin has been described to play a role in developmental and/or adult tissue homeostasis.

(McMahon et al., 1998). In the medial somites noggin expression is promoted by Wnt-1, which in turn antagonizes BMP-4 activity and accelerates myogenesis (Hirsinger et al., 1997).

Noggin is also critical for embryonic chrondrogenesis, osteogenesis and joint formation (Fig. 2) (Gong et al., 1999; Tylzanowski et al., 2006). Noggin expression in osteoblasts is augmented in the presence of BMP-2, -4, -5, -6, -7 (Gazzerro et al., 1998; Song et al., 2010). In chrondrocytes it has been described that Indian hedgehog induces noggin expression (Pathi et al., 1999). Transgenic mice overexpressing noggin in mature osteocalcin-positive osteoblasts revealed a dramatic decrease in bone mineral density and bone formation rate due to impaired osteoblast recruitment and function (Wu et al., 2003). Mouse studies have furthermore shown that noggin is expressed postnatally in the mesenchyme of patent, but not fusing, cranial sutures where noggin transcripts are down-regulated by fibroblast growth factor-2 (FGF-2) and FGF-9. It is speculated that premature cranial suture fusion caused by constitutive activation of FGF receptors is the result of inappropriate inhibition of noggin expression (Warren et al., 2003).

4. Biological function

With the growing knowledge on BMPs and their respective tissue targets, the understanding of noggin's functionality is also strengthened (Table 1). In noggin null mice augmented BMP activity evokes a series of developmental abnormalities which include failure of neural tube formation, hair-follicle retardation, dysmorphogenesis of the axial skeleton and joint lesions (McMahon et al., 1998; Tylzanowski et al., 2006). Since noggin null mice are embryonic lethal, the role of noggin in adult tissue homeostasis is mostly undetermined.

How important noggin is in humans has been highlighted through the discovery of heterozygous missense mutations of *NOG* with effect on joint morphogenesis, indicating functional haploinsufficiency (Marcelino et al., 2001; Gong et al., 1999). Thereby, increased noggin activity results in skeletal dysplasia such as proximal symphalangism (SYM1) and multiple synostosis syndrome 1 (SYNS1) (Marcelino et al., 2001).

Beyond that, it has recently been shown that noggin in combination with basic fibroblast growth factor (bFGF) is sufficient to maintain prolonged growth of human embryonic stem cells (hES) *in vitro* (Wang et al., 2005). Additionally, noggin also antagonizes BMP signaling to regulate the stem cell niche during neurogenesis (Lim et al., 2000).

A novel role of noggin in osteolytic prostate cancer cells has been recently implicated. Thereby it was found that noggin expression is restricted to cell lines that induce osteolytic bone metastases. Re-expression of noggin in prostate cancer cells reduced the osteosclerotic capacity and normalized the overall bone structural environment and balanced bone remodeling (Schwaninger et al., 2007).

5. Possible medical and industrial applications

5.1. Noggin's affinity to BMPs

Since Groppe and co-workers published the crystal structure of the BMP-7/noggin complex, a series of noggin point mutations was engineered to evaluate their respective binding affinities to BMP-7. Three noggin mutations (L46D, E48K and I218E) revealed lower BMP-7 binding affinities compared to wild type noggin, leading *vice versa* to the idea of tailored noggin-insensitive and thus more potent BMPs (Fig. 1) (Groppe et al., 2002). Since the binding sites for BMP type I and BMP type II receptors and noggin overlap on the surface of BMPs, the identification of BMP specific residues involved in binding to noggin but not to BMP receptors is a critical determinant and still not resolved.

5.2. Susceptibility of BMPs to noggin

Recently, two independent groups demonstrated that single amino acid changes in BMPs can determine the susceptibility to

noggin inhibition which provides insight on noggin's ligand selectivity. Mutations of BMP-14 at position N64 (N64K and N64T), which are linked to patients with SYM1, protects the ligand from noggin antagonism with subsequent elevated cartilage production in an *in vivo* chicken model (Fig. 1) (Seemann et al., 2009). A comparative analysis of BMP-6 and its close paralogue BMP-7 demonstrated that a single amino acid change at position K60 (BMP-6) to E60 (BMP-7) is mediating increased resistance to noggin inhibition, thereby explaining its strong osteo-inductive properties (Song et al., 2010). Interestingly, the SYM1 associated mutations result in overactive BMP-14 due to altered BMP type I receptor binding specificity whereas the BMP-7/E60 noggin complex is suggested to interfere with BMP receptor type II binding (Fig. 1) (Song et al., 2010; Seemann et al., 2009).

A systemic analysis of a panel of BMPs revealed that crucial amino acids for the determination of noggin's susceptibility are conserved among BMPs which enables a prediction on noggin's effect on yet not investigated BMP proteins. Both groups show that it is possible to engineer BMP variants, which overcome the noggin regiment through amino acid substitutions. That allows for development of more effective, second generation BMP proteins (e.g.: BMP-2/P36K; BMP-7/K60E; BMP-14/N64K and N64T) with potential clinical applicability in spinal fusion, long bone non-union fracture treatment and osteoarthritis (Fig. 1) (Song et al., 2010; Seemann et al., 2009).

5.3. Noggin's bioavailability

Additionally, it has been described that noggin contains a central, highly basic heparin-binding segment that facilitates binding and storage in the extracellular matrix (ECM) (Paine-Saunders et al., 2002). Thereby, ECM bound noggin plays an essential role in the formation of lateral BMP gradients, a prerequisite for cell polarization, embryogenesis and tissue patterning (Fig. 2). In fact, an area of ongoing research investigates the regulatory capacity of bone matrix changing with environmental entities (e.g.: illness, aging) which might interfere with the BMP and noggin interplay. Recently, noggin mutants lacking the heparin binding domain (hNogΔB2) where engineered. Thereby, systemic administrated hNogΔB2 gave rise to reduced binding to heparin sulphate proteoglycans and revealed improved bio-availability/–activity, thus representing a potential candidate for gene therapy (Paine-Saunders et al., 2002).

Even though the detailed functionality of noggin is starting to be elucidated, this review indicates that there are still a lot of gaps to fill in order to get a complete view on noggin's action. In the future it will be interesting to see whether noggin can act independently of BMPs via binding to its own receptor, as it has been recently shown for the binding of gremlin to the VEGF receptor-2 (Mitola et al., 2011). Thereby the role of noggin on BMPs in endoderm derivates and the regulation of *in vivo* adult tissue homeostasis will be particularly interesting.

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