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Role of nerve growth factor (NGF) and its receptors in folliculogenesis

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Summary

Nerve growth factor (NGF) is a prototype member of the neurotrophins family and has important functions in the maintenance of viability and proliferation of neuronal and non-neuronal cells, such as certain ovarian cells. The present review highlights the role of NGF and its receptors on ovarian follicle development. NGF initiates its multiple actions through binding to two classes of receptors: the high affinity receptor tyrosine kinase A (TrkA) and the low-affinity receptor p75. Different intracytoplasmic signalling pathways may be activated through binding to NGF due to variation in the receptors. The TrkA receptor activates predominantly phosphatidylinositol-3-kinase (PI3K) and mitogenic activated protein kinase (MAPK) to promote cell survival and proliferation. The activation of the phospholipase type Cγ (PLCγ) pathway, which results in the production of diacylglycerol (DAG) and inositol triphosphate (IP3), culminates in the release of calcium from the intracytoplasmic cellular stocks. However, the details of activation through p75 receptor are less well known. Expression of NGF and its receptors is localized in ovarian cells (oocyte, granulosa, theca and interstitial cells) from several species, which suggests that NGF and its receptors may regulate some ovarian functions such as follicular survival or development. Thus, the use of NGF in culture medium for ovarian follicles may be of critical importance for researchers who want to promote follicular development *in vitro* in the future.

Keywords: p75, Follicles, NGF, Ovary, TrkA

Introduction

Previously researchers have described growth factors that control the regulation of neural cell development and differentiation. These factors also participate in regulation of processes in non-neuronal cells (Dissen *et al.*, 2002). Neurotrophic factors, for example, demonstrate this double activity and contribute to the development of a variety of non-neural tissues, including those within the pancreas, thymus, heart, adenohypophysis or ovaries (Ojeda & Dissen, 1994; Tessarollo, 1998).

Neurotrophic factors are divided into two main families: the neurotrophins (NT) and glia-derived neurotrophic factor (GDNF; Airaksinen & Saarma, 2002; Chao, 2003). The NT family consists of some peptides with a high degree of structural homology, whose main components are nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin-4/5 (NT-4/5; Ibañez, 1998; Aloe, 2004; Dissen *et al.*, 2009).

The reproductive aspect of NTs, especially NGF, have been studied widely; they are present in the ovaries and knockout of their genes impairs both follicular formation and development (Dissen *et al.*, 2002). However, it is not known if NGF functions only

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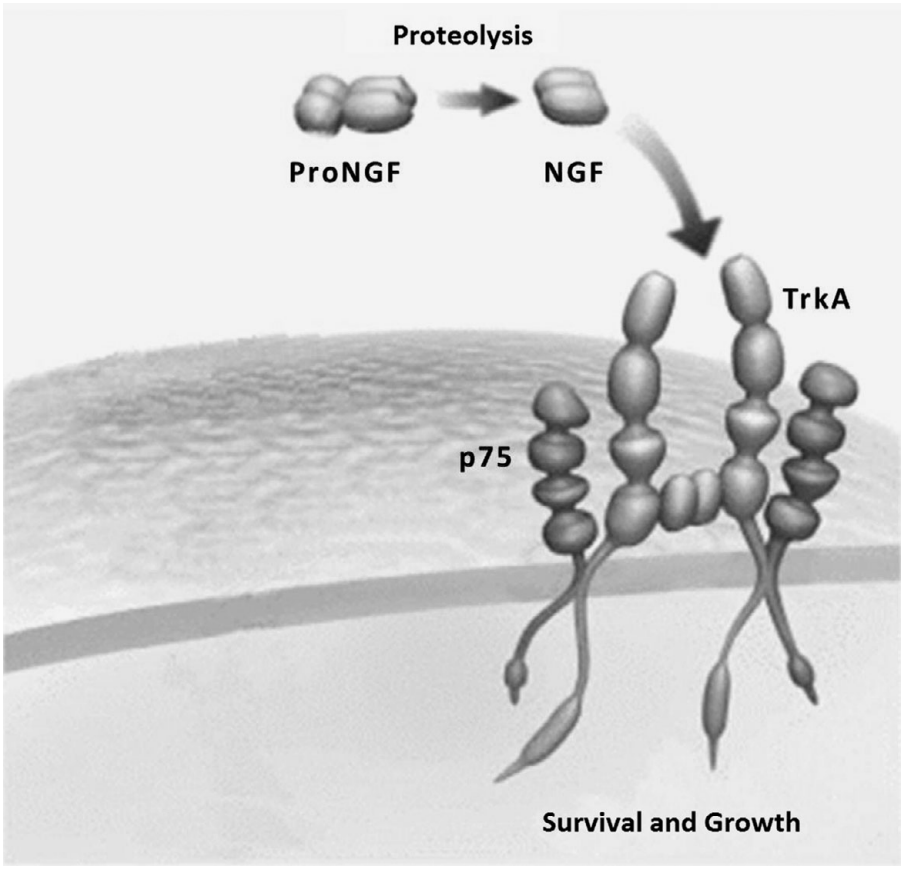

Figure 1 Schematic model that illustrates high (TrkA) and low (p75) affinity receptors for NGF. Adapted from GrandPré *et al.* (2002).

in the ovary, despite its evident action as a trophic support in the sympathetic innervation of the organ and its important role during ovarian and follicle development (Mayerhofer *et al.*, 1997; Dissen *et al.*, 2001). This review describes the role of NGF and its receptors in ovarian follicular development.

Structural characterization of NGF and its receptors

NGF is a prototype glycoprotein that belongs to the NT family. It is synthesized initially as pro-NGF (306 amino acids) that is cleaved and translocated to the rough endoplasmic reticulum, then cleaved by extracellular proteases to produce a biologically active protein (118 amino acids). The mature NGF is thus a covalent homodimer with a molecular weight of 130 kDa (Covaceuszach *et al.*, 2004; Mouri *et al.*, 2007; Nomoto *et al.*, 2007).

The tridimensional structure of NGF was revealed by McDonald *et al.* (1991), who verified the structure using X-ray crystallography as having an elongated shape with a core formed by twisted beta sheets and bound by disulfide bridges. The molecule is made up of three subunits (α, β and γ complex). The β-NGF subunit is responsible for the biological activity and the γ-NGF subunit is a highly specific active protease (26-kDa serine protease of the kallikrein protease group) and is responsible for the conversion of the pro-NGF transcript into its active form. The function of the α-NGF subunit is not known and it appears to be inactive (Sofroniew *et al.*, 2001). During the activation of NGF, the γ-NGF subunit proteins are hydrolyzed to enable the conversion of pro-NGF synthesized from the gene into the active homodimer NGF. In this process, the protein remains stable and there is no segregation of α-NGF and γ-NGF subunits. It is believed that although this protein has not shown biological function, it may serve to protect NGF from proteolytic enzymes present in tissues (Levi-Montalcini & Calissano, 1986).

NGF begins its biological role by binding to two known receptors (Fig. 1). One of these shows high affinity to NGF and is called tyrosine kinase receptor A (TrkA) (Ibañez, 1998; Terenghi, 1999). The other is a low-affinity receptor called neurotrophin

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receptor p75 ($p75^{NTR}$ or p75). NGF signalling occurs preferentially through the TrkA receptor (Kaplan *et al.*, 1991), whereas p75 may potentiate or inhibit the biological responses mediated by TrkA (Kohn *et al.*, 1999). Phylogenetic analysis of NGF and its TrkA receptor identified co-evolution between these loci, and increased the receptor–ligand specificity (Halböök *et al.*, 1998).

The *TrkA* gene maps to chromosome band 1q22 (Valent & Bernheim, 1997) and contains 17 exons that codify a transmembrane protein of 140 kDa, which has an extracellular binding domain (composed of three leucine-rich motifs flanked by two cysteine clusters, two immunoglobulin-like C2 type domains – Ig-C2), a single transmembrane domain and an intracellular domain that has tyrosine kinase activity (Greco *et al.*, 1996). NGF binding to the extracellular domain leads to receptor oligomerization and this rearrangement allows the domains of the closest kinases in the receptor chain to phosphorylate one another, in a process called autophosphorylation (Kaplan & Miller, 2000; Alberts *et al.*, 2004). The activation of TrkA receptors begins the signalling cascades, including the intracellular pathways.

The receptor p75 is a member of the death-promoting tumour necrosis factor receptor (TNF-R) superfamily, which also includes the characteristic death receptors TNF-R apoptosis-inducing ligand (TRAIL)-R and Fas/CD95 (Haase *et al.*, 2008). p75 is a 75 kDa glycoprotein with four extracellular cysteine-rich repeats that are required for ligand binding (Chao, 2003; Barker, 2004). It is a single pass type I transmembrane receptor, with an intracellular domain that contains a juxtamembrane region and a type II consensus death domain (DD) sequence (Roux & Barker, 2002). Although p75 binds dimeric neurotrophin ligands, there is some controversy over the oligomeric status of p75 and evidence indicates that it may also signal as a monomer or as a dimer (Vilar *et al.*, 2009). Recently, it was shown that p75 can form covalent homodimers through a disulphide bond in the transmembrane region. In this case, neurotrophin binding is understood to induce a conformational change in the receptor, such that it pivots on the disulfide bond at Cys257 to permit access of intracellular adaptor proteins to the intracellular domain in what is termed a 'snail-tong model' (Simi & Ibañez, 2010). The receptor does not possess intrinsic enzymatic activity and instead transduces signals through recruitment of a variety of adaptor proteins to the intracellular domain, thus leading to proliferation, survival, or cell death (He & Garcia, 2004). Interestingly, p75 also serves as a receptor for immature pro-neurotrophins, which induce cell death in a manner that is dependent on binding to the co-receptor sortilin (Nykjaer *et al.*, 2004).

Cellular signalling through NGF/receptor to regulate folliculogenesis

The effects of NGF are related to the activation of different biochemical pathways (Fig. 2), activation of the MAPK and PI3K pathway and inactivation of the apoptotic proteins Bad and Bax from the Bcl-2 family, both pathways are activated by the TrkA receptor. Previous researchers have also described the activation of the PLCγ pathway that produces DAG and IP3, which induce the release of calcium from the intracytoplasmic cellular stocks (Glebova & Ginty, 2005). Finally, activation of the small GTPase Ras in response to NGF has been shown and linked to cell survival and differentiation (Caporali & Emanueli, 2009).

The details of activation performed by the p75 receptor are less well known, but it may result in the production of ceramides, or activation of nuclear factor-kappa B (NF-κB) or regulator kinases such as c-Jun N-terminal kinase (JNK; Lee *et al.*, 2001; Chao, 2003). p75 signalling can also lead to downstream activation of NF-κB, which promotes cell survival through upregulation of anti-apoptotic genes such as *cFLIP* and this event interferes with the activation of initiator caspase-8, the Bcl-2 family member Bcl-XL, and inhibitor of apoptosis proteins (IAPs) XIAP and cIAP1/2 (Karin, 2006; Baud & Karin, 2009). Together with the pro-apoptotic effects of p75, these findings show that p75 can act as a bifunctional switch to direct the cell down opposing pathways of cell death or survival (Molloy *et al.*, 2011).

Thus, the p75 receptor shows an independent role in apoptosis that may be anti- or pro-apoptotic (Chao, 2003). The anti-apoptotic stimulus begins with the activation of a ceramide-dependent signalling pathway (Shi *et al.*, 2004). In this case, NGF interacts with the p75 receptor to hydrolyze sphingomyelin, which results in the production of an apoptosis cascade signalling inhibitor called ceramide (Barrett, 2000). The pro-apoptotic stimulus induced by p75 follows the intrinsic apoptotic pathway, with the release of cytochrome *c* by mitochondria and activation of caspase-9 (Bhakar *et al.*, 2003). However, cell apoptosis mediated by p75 does not occur when high affinity receptors (TrkA) are also expressed (Botchkarev *et al.*, 2000).

Ras/MAPK pathway

Activation of the Ras protein occurs after NGF binds to its cellular receptor TrkA, which is needed for cell differentiation and survival. This process is mediated through an Shc adaptor molecule that binds to phosphorylated tyrosine 490, which is critical

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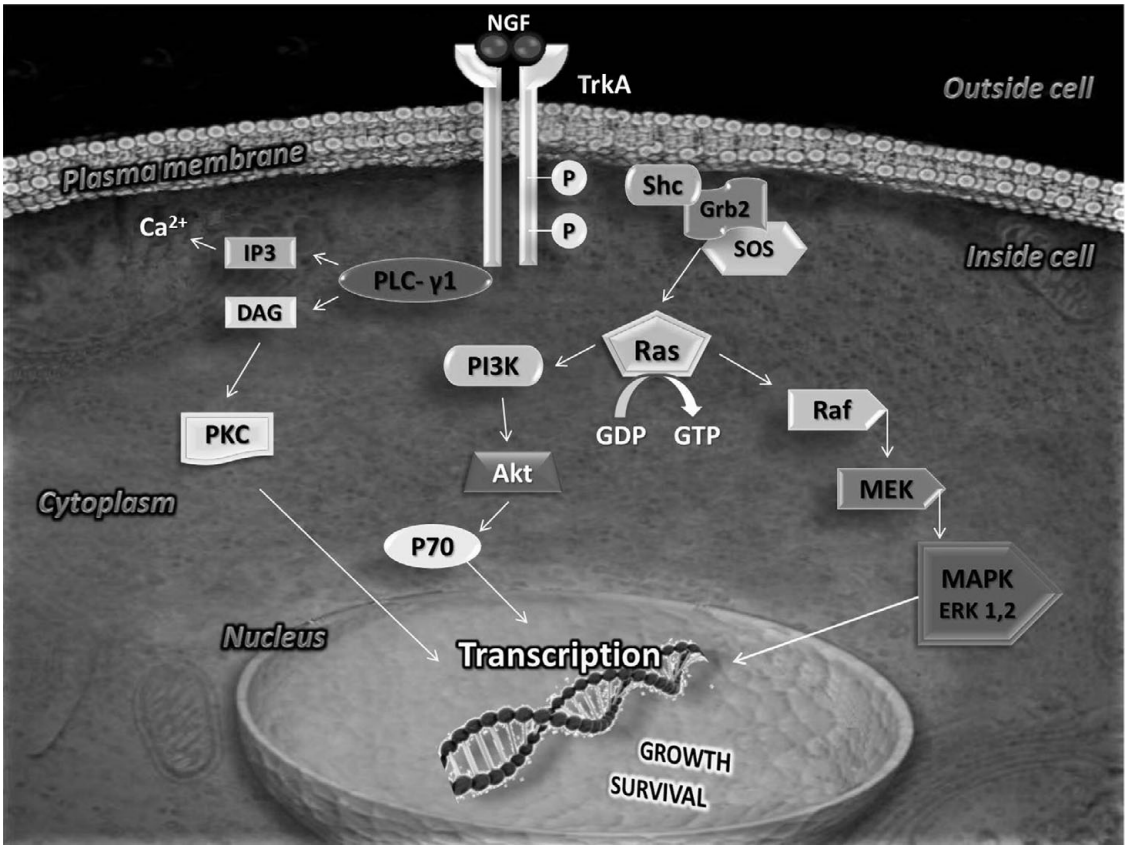

Figure 2 Schematic representation of signalling pathways between NGF and TrkA. NGF binds to its receptor TrkA, leading to dimerization and autophosphorylation. The Shc adaptor molecule binds to the phosphate in the receptor and to the Grb2–SOS complex. The Ras complex activates, exchanging the GDP for GTP. Activated Ras interacts directly with the Raf protein, which leads to the sequential activation of MEK and MAPK. MAPK translocate to the nucleus, where it phosphorylates transcriptional factors, promoting cellular differentiation. Ras also promotes PI3K activation, which activates substances responsible for follicular survival and growth. Activation of PLCγ1 results in the release of Ca^{2+} and PKC activation.

for the activation of the Ras signalling cascade. Shc is recruited to its recognized site in activated Trk by interactions with the Shc PTB domain (Nimnual *et al.*, 1998). Shc phosphorylation produces a site on phosphotyrosine Shc that recruits other adaptor proteins that contain the SH2 domain, such as growth factor receptor-bound protein-2 (Grb2), which binds to the exchange factor of the nucleotide guanine (SOS – son of sevenless; Robinson *et al.*, 2005). The Grb2–SOS complex is translocated to the plasma membrane, where SOS activates a small G protein p21ras and promotes the transition from inactive Ras–GDP to active Ras–GTP (guanosine triphosphate; York *et al.*, 2000). The activated Ras protein stimulates signalling through several cytoplasmic kinase proteins, such as Raf (kinase specific for serine/threonine), MEK (MAPK kinase activator) and MAPK (Wood *et al.*, 1992; Xing *et al.*, 1998). The activation of one or more kinases leads to phosphorylation and activation of MEK and isoforms of MAPK, ERK-1 and ERK-2 (kinases regulated by an extracellular signalling -1/-2) (Crews *et al.*, 1992; Wood *et al.*, 1992). MAPK translocates to the cell nucleus, where it phosphorylates a group of molecules responsible for transcription, thus beginning cellular proliferation (Silva *et al.*, 2009).

PI3K and PLCγ1 pathways

One of the main signalling mechanisms that involves lipids is the cleavage of the membrane phosphatidylinositol to form DAG and IP3 by PLCs. IP3 may bind to its receptors in the rough endoplasmic reticulum, releasing Ca^{2+} from these stocks, as shown previously, while DAG may activate several isoforms of protein kinase Cs (PKCs). Therefore, PI3K and PLCγ act as intracellular messengers, whose function is to transmit the membrane receptor signalling to several proteins that will make this sign noticeable by the cell (Lenz, 2000).

Considering that PI3Ks may be activated by Ras (Rodriguez-Viciana *et al.*, 1994) and by the subunits βγ of the protein G (Lopez-Ilasaca *et al.*, 1997), these enzymes represent an important connection point

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between the signalling activators of protein G, Ras and PKC and their numerous substrates (Lenz, 2000).

The Ras independent pathway occurs when the GAB1 adaptor proteins (GRB2-associated binding protein-1) and Shc are phosphorylated by TrkA. As soon as it is activated, they are associated with Grb2 to produce a complex that activates PI3K (Holgado-Madruga *et al.*, 1997). However, Trk phosphorylation can also promote phosphorylation of the insulin 1 receptor, which recruits and activates PI3K (Yamada *et al.*, 1997). Once activated, either by Ras or by its independent pathway, PI3K phosphorylates several proteins important for this process: Akt (also known as kinase B protein), the RAC-PK and the ribosomal protein p70 S6 kinase (p70S6K or p70) (Chung *et al.*, 1994; Marte & Downward, 1997). Akt is considered to be the most important protein phosphorylated by PI3K, and controls the activity of several other proteins that are important for cellular survival. This process occurs through the regulation of proteins that control the activity of certain transcriptional factors that promote apoptosis (Brunet *et al.*, 2001), such as the BAD cascade (a pro-apoptotic member of the Bcl-2 family; Yuan *et al.*, 2001).

PI3K has a role in cellular growth and differentiation through phosphorylated phosphatidylinositol that recruits the guanine exchange factors Cdc42/Rho/Rac (GEF), which act on the organization of actin filaments into cytoskeleton, and improves the orientation of mitotic fusion (Yuan *et al.*, 2003).

The activation of PLCγ by Trk promotes an increase in intracellular Ca^{2+} levels and the regulation of kinase C protein. These proteins activate numerous intracellular enzymes, which include all isoforms of kinase C protein and calcium-/calmodulin-dependent protein kinase C (CaMKII), which then activates other important proteins. Other such important proteins are MEK and ERK1/ERK2 (Corbit *et al.*, 1999).

Other Ras-independent pathways have been related to cellular survival and differentiation. Among these, the phosphorylation of the SNT molecule by NGF (Rabin *et al.*, 1993) can be highlighted. In this case, once phosphorylated, SNT is translocated into the nucleus, where it acts as a transcription factor by regulating the genes that control cell cycle (Rabin *et al.*, 1993).

Expression of NGF and its receptor in the ovary

The expression of NGF and its receptors (TrkA and p75) was verified in ovarian cells (oocyte, granulosa, theca and interstitial cells) of several species, including human (Spears *et al.*, 2003; Salas *et al.*, 2006), rodent (Romero *et al.*, 2002; Shi *et al.*, 2004), bovine (Dissen *et al.*, 2000; Levanti *et al.*, 2005), ovine (Barboni *et al.*, 2002) and caprine (Ren *et al.*, 2005). Thus, NGF and its receptors regulate several functions in the ovary, such as sexual development (Lara *et al.*, 1990), follicular development and ovulation (Dissen *et al.*, 1996; 2001) through autocrine and paracrine ways.

Levanti *et al.* (2005) verified a weak immunostaining for the protein p75 in the ovarian stromal in bovine by immunohistochemistry and a high immunoreactivity in the oocytes of preantral follicles (primordial, primary and secondary) and early antral follicles (tertiary). Immunoreactivity for the TrkA receptor was found in the oocyte, granulosa and theca cells, and appeared independent of the follicular maturation stage. In swine ovaries, the primordial and primary follicles showed positive immunostaining for p75 and TrkA only in granulosa cells, while in tertiary follicles, this same receptor was found both in follicular cells and oocytes. In addition, a weak immunoreactivity was found in stromal cells and high immunoreactivity in corpus luteum cells (Levanti *et al.*, 2005).

The TrkA receptor protein was localized in the oocytes of mouse primordial follicles and visualized using immunofluorescence, (Dissen *et al.*, 2001), while the NGF ligand was expressed in the theca and granulosa cells of preantral and antral follicles in rat ovaries (Dissen *et al.*, 1991). The expression pathways found in the oocytes and somatic cells indicated that NGF is a potential factor that can regulate intra-oocyte activation of PI3K (Adhikari & Liu, 2009). Noticeable amounts of NGF were also detected in the follicular fluid of rat antral follicles (Barboni *et al.*, 2002), and probably originated from granulosa and theca cells (Dissen *et al.*, 2001).

In addition, other immunocytochemical studies revealed that the p75 receptor protein was expressed in the mesenchyme cells in the rat fetal ovary (Dissen *et al.*, 1995; Ojeda *et al.*, 2000). In hamsters, the staining for NGF and its receptors (TrkA and p75) was detected in the oocyte, granulosa, theca, interstitial and luteinic cells of all follicular categories (Shi *et al.*, 2004). In mouse, TrkA was localized in the oocytes, granulosa and theca cells of primary and secondary follicles, while the p75 receptor was localized only in interstitial cells (Dissen *et al.*, 2001; Weng *et al.*, 2009). In *Rhesus* monkey ovaries, detection of p75 receptor mRNA expression by immunocytochemistry indicates that this organ is able to synthesize this receptor (Dees *et al.*, 1995).

Role of NGF on follicular development

Survival

Some studies have demonstrated that NTs and their receptors play an important role in the development

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of the mammal ovary, oogenesis, folliculogenesis and embryo development (Bjorling *et al.*, 2002; Krizsan-Agbas *et al.*, 2003; Shi *et al.*, 2004; Ren *et al.*, 2005). Among these, NGF is highlighted because of its role in follicular survival.

NGF is a protein necessary for the maintenance, survival and development of the neuronal population in the central and peripheric nervous system (Angeletti & Bradshaw, 1971; Levi-Montalcini, 1987; Snider, 1994). In the ovaries, NGF also acts as a trophic support in the sympathetic innervation of this organ, which is important for folliculogenesis (Dissen *et al.*, 2001). The direct action of NGF on follicular survival was demonstrated by Chaves *et al.* (2010). These authors cultivated caprine preantral follicles enclosed in fragments of ovarian tissue in the presence of NGF, and demonstrated the ability of NGF in maintainance of *in vitro* follicular survival and ultrastructure in a dose-dependent way. In humans, culture of fetal ovaries of 13–16 weeks of age in the presence of a Trk inhibitor resulted in a decrease in oogonia survival (Spears *et al.*, 2003), which demonstrated that NGF has a fundamental role in the maintenance of survival and that the receptor that acts in this signalling pathway is Trk. Moreover, analysis of NGF expression and its receptors in antral follicles during the estrous cycle demonstrates that atretic follicles in the proestrus phase showed a higher level of p75 expression than at others days of the cycle (Shi *et al.*, 2004).

In addition to direct action, NGF may act indirectly in follicular survival through the production of biologically active follicle-stimulating hormone receptors (FSHR) (Salas *et al.*, 2006). Rats ovaries treated with NGF developed the capacity to response to follicle-stimulating hormone (FSH), with the formation of cAMP in preantral follicles (Romero *et al.*, 2002). Similar results were obtained in human cells, in which the culture of granulosa cells with NGF also increased expression of the FSHR in these cells (Salas *et al.*, 2006). As FSH is a gonadotrophin that acts as a survival factor in the culture of preantral follicles (Matos *et al.*, 2007), we can assume that any substance, such as NGF, that increases the number of FSHRs is important.

However, high concentrations of NGF may be harmful to the female reproductive lifespan, as it may reduce fertility (Dissen *et al.*, 2009). This fact was confirmed recently in the ovaries of transgenic animals with the excessive production of NGF, which caused an increase in the apoptosis rate of granulosa cells, due to the overproduction of the protein stathmin (STMN1) within the ovaries. In the phosphorylated stage, this protein is expressed in granulosa cells and is responsible for the intermediation of a cell death signal initiated by tumour necrosis factor α (TNFα). Researchers also observed an increase in TNFα synthesis in transgenic animals and the blockage of the phosphorylation of STMN1 protein by tyrosine kinase receptors. Thus, inhibition of TNFα actions *in vivo*, through the administration of a soluble TNFα receptor, blocked the increase in phosphorylated STMN1 production, as well as apoptosis of granulosa cells in the ovaries of these animals (Garcia-Rudaz *et al.*, 2011). Moreover, in transgenic animals that have excessive production of NGF, there is a tendency for the formation of ovarian cysts (Dissen *et al.*, 2009). This predisposition occurs due to the high production of 17α-hydroxyprogesterone, testosterone and estradiol in response to gonadotrophins, especially high levels of luteinizing hormone (LH; Garcia-Rudaz *et al.*, 2011).

Activation of primordial follicles

After the organization of oocytes and somatic cells in primordial follicles, the newly formed follicles pass through a differentiation process in which flattened pre-granulosa cells located around the oocyte acquire a cuboidal morphology, in a process called follicular activation (Hirshfield, 1991; Fahnestock *et al.*, 2004). NGF and its receptors seem to be involved in this process, as NGF is present in the follicles at the primordial stage in rats. Studies with NGF knockout mice showed that these animals had a reduced number of primary and secondary follicles (Dissen *et al.*, 2001, 2002; Romero *et al.*, 2002). The absence of NGF promoted a reduction in the proliferation rate of mesenchyme somatic cells before the formation of primordial follicles, detected *in vivo* and *in vitro*, and consequently led to an increase in the number of oocytes that are not involved with somatic cells to form primordial follicles (Ojeda *et al.*, 2000). In addition, functional analysis using neonate ovaries in *in vitro* culture systems have confirmed that an increase in NGF promotes an increase in the activation rate of primordial follicles (Paredes *et al.*, 2004).

NGF seems to interact with other growth factors, such as growth and differentiation factor-9 (GDF-9) and kit ligand (KL; Oktay *et al.*, 1995), at the start of the pre-granulosa differentiation and cellular growth in primary follicles. Another indication of the NGF role in follicular activation is the inhibition of the TrkA receptor, which is related to a reduction in the number of developing follicles. Granulosa cells of rat primary follicles showed a higher expression of TrkA than those of more developed follicles, which indicated that NGF seems to be most important in early follicle development. However, in bovine, the expression of this factor remained at constant levels throughout all folliculogenesis (Dissen *et al.*, 2000).

Compared with the NGF knockout, rats with mutation of the p75 receptor showed a normal population of primordial, primary and secondary follicles (Lee *et al.*, 1992; Ojeda *et al.*, 2000). Moreover,

the ovaries of p75 knockout mice showed an increase in the number of primary and secondary follicles (Ojeda *et al.*, 2000). Thus, the results suggested that p75 may act as a modulator in pre-thecal mesenchyme cells, and regulate follicle activation.

It is interesting to note, however, that no effect of NGF (1, 10, 50, 100 and 200 ng/ml) was observed in the transition from primordial to primary follicles in caprine (Chaves *et al.*, 2010). This fact was confirmed previously by Nilsson *et al.* (2009), in which the ovaries treated with 50 ng/ml of NGF did not show any effect in the transition from primordial to primary follicles in rats. One hypothesis that could explain this result would be the presence of stimulatory substances for the activation of primordial follicles, such as insulin, in the basic medium.

Follicular growth

Cellular growth is related to the ability to promote proliferation of mesenchyme and follicular cells, as well as to induce FSHR synthesis (Romero *et al.*, 2002; Salas *et al.*, 2006). In this aspect, NGF has been associated with follicular growth because it shows mitogenic effects in several types of non-neural cells, including mesenchyme cell lines (Cordon-Cardo *et al.*, 1991; Dissen *et al.*, 2000; Sortino *et al.*, 2000) and epithelial cells (Garcıa-Suarez *et al.*, 2000).

In a previous study (Dissen *et al.*, 2001), researchers observed that secondary follicle development is reduced in *NGF* gene knockout mice. Using immunohistochemical techniques for the detection of proliferation cell nuclear antigen (PCNA) and bromodeoxyuridine (BrdU), there is a reduction in the proliferation rate of a mesenchyme cell line and epithelial cells within the ovaries of these mice, respectively. One explanation is that there may be an involvement of Trk receptors that facilitates the effect of NGF on the somatic cell proliferation in the ovary (Dissen *et al.*, 1996).

The growth factors of the TGF-β superfamily, which are produced by the mesenchyme cells in the ovary, may be among other potential factors that interact with NGF for follicular growth. They also regulate the growth and differentiation of follicular cells and of the ovary (Skinner *et al.*, 1987; Gitay-Goren *et al.*, 1993), facilitating FSH-dependent events, such as aromatase activity (Bendell & Dorrington, 1988), steroidogenesis (Dodson & Schomberg, 1987) and the formation of LH receptors (Kim *et al.*, 1994).

Although some studies indicate mitogenic effects of NGF in caprine, NGF did not promote follicle and oocyte growth after 7 days of culture within ovarian fragments (Chaves *et al.*, 2010). This discrepancy may be attributed to the culture conditions utilized such as the concentration of NGF, culture period, or differences between the species or cellular types.

NGF also has the indirect capacity to induce angiogenesis in several tissues, such as the skin (Chiaretti *et al.*, 2002), skeletal muscle (Emanueli *et al.*, 2002), cornea (Seo *et al.*, 2001) and central nervous system (Calza *et al.*, 2001) through the stimulus of vascular endothelial growth factor (VEGF) production (Julio-Pieper *et al.*, 2009). Studies revealed that VEGF, besides being a potent mitogenic factor, also has an important role in the regulation of the vascular structure and in the increase in capillary permeability (Redmer *et al.*, 2001). Moreover, Bruno *et al.* (2009) showed that VEGF acts on follicular survival and development, to promote an increase in follicle and oocyte diameters in caprine preantral follicles. However, these findings also indicate that NGF may participate in ovarian disturbances, such as polycystic ovaries, ovarian tumours and ovarian hyperstimulation syndrome, all subjacent of ovarian angiogenesis (Agrawal *et al.*, 1998; Ludwig *et al.*, 1999; Albert *et al.*, 2002).

Final consideration

NGF was found 50 years ago as a molecule that promoted the survival and differentiation of sympathetic and sensory neurons. Its role in neuronal development has been characterized extensively, but recent findings suggest an unexpected diversity of NGF actions in other organs, such as the ovary. Studies have demonstrated the essential role of NGF in mammal folliculogenesis both in a direct way, such as through the nervous stimulus in the ovary, or by its indirect role, through mesenchyme cells (theca cells).

The complete understanding of NGF actions within the ovaries is still unclear due to the great variety of kinases that may be expressed and also by the lack of studies on this growth factor in the ovary. Thus, the use of NGF in the media used for culture *in vitro* of ovarian follicles may be of fundamental importance to promote follicular development.

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