

ADAM17 As Inducer of Male Germ Cell Apoptosis

ADAM17 como Inductor de la Apoptosis de Células Germinales Masculinas

Ricardo D. Moreno*

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SUMMARY: Apoptosis during mammalian spermatogenesis is important to control sperm output and to eliminate damaged unwanted cells. A deregulation in apoptosis rate has deleterious consequences and leads to low sperm production. Apoptosis in spermatogenesis has been widely studied, but the mechanism by which it is induced under physiological or pathological conditions has not been clarified. We have recently identified the metalloprotease ADAM17 (TACE) as a putative physiological inducer of germ cell apoptosis. ADAM17 is widely distributed enzyme in many tissues, its main function is to shed (release) its substrates, which leads to the activation of different signaling pathways according to the cellular context. However, in spermatogenesis, ADAM17 is involved in the control or induction of germ cell apoptosis, this seems to be unique and may reflect the complexity of spermatogenesis. In this review I will focus on the published evidence that show how this enzyme involved in the control of germ cell apoptosis during physiological condition and in different insults such as heat stress, DNA damaging agent or endocrine disruptors.

KEY WORDS: ADAM17; Apoptosis; male germ cell.

INTRODUCTION

The aim of a mature mammalian spermatozoa is to reach the oocyte and then fuse with it in order to form a new zygote; this process is known as fertilization. Mammalian spermatozoa are formed in the testis and their production relies on physiological and environmental factors, which may attenuate, increase or even totally suppress testicular function. Germ cell apoptosis has been show to play an important role in controlling sperm output in many species, and its de-regulation has been related to infertility (Lee *et al.*, 1997, 1999, 2009; Feng *et al.*, 1999; Weikert *et al.*, 2004; Moreno *et al.*, 2011). Recent reports have shown than global human sperm production is decreasing, which may be is caused by environmental toxicants that induce germ cell apoptosis (Sinawat, 2000). In the same way, modern life style and use of underwear has been proposed to produce an elevated scrotal

temperature that may cause germ cell apoptosis, akin to transient heat stress (43 °C for 15 min) in testis (Lizama *et al.*, 2009; Reyes *et al.*, 2012). Many studies have shown the relevance of apoptosis in regulating spermatozoa output and eliminating damaged germ cells, particularly spermatocytes (Beumer *et al.*, 1998; Allemand *et al.* 1999; Feng *et al.*; Elliott *et al.*, 2010). Even more, in seasonal breeding mammals, apoptosis plays an important role in the massive germ cell depletion observed during the non-breeding season (Comitato *et al.*, 2006). Finally, a massive germ cell death occurs in physiological conditions (constitutive apoptosis) during the first round of spermatogenesis, which is vital in order to establish a proper interaction between germ and Sertoli cells and to eliminate unwanted germ cells (Oakberg, 1956; Rodriguez *et al.*, 1997; Moreno *et al.*, 2006) Different experimental approaches

* Departamento de Fisiología, Pontificia Universidad Católica de Chile, Santiago, Chile.

have pointed out that germ cells undergoing meiosis (spermatocytes) are the main target for apoptosis, and a smaller fraction of spermatogonia (Oakberg; Rodriguez *et al.*, 1997; Jahnukainen *et al.*, 2004; Moreno *et al.*). Therefore, apoptosis is important in physiological and pathological conditions, and the knowledge of the molecular mechanisms underlying this process may help to develop new pharmacological tools in order to modulate fertility.

Apoptosis: The basics

Apoptosis is a physiological process for killing cell and critical for the normal development and function of multicellular organisms (Rodriguez *et al.*; Tanuma, 1999; Riedl & Shi, 2004). Abnormalities in cell death control contribute to a variety of diseases, including cancer, autoimmunity, and degenerative disorders. Signaling for apoptosis occurs through multiple independent pathways that are initiated either from triggering events within the cell or from outside the cell, for instance, by ligation of death receptors (Squier & Cohen, 1996; Giammona *et al.*, 2002; Coureuil *et al.*, 2006; Ott *et al.*, 2007; Degterev & Yuan, 2008). All apoptosis signaling pathways converge on a common machinery of cell destruction that is activated by a family of cysteine proteases (caspases) that cleave proteins at aspartate residues. Dismantling and removal of doomed cells is accomplished by proteolysis of vital cellular constituents, DNA degradation, and phagocytosis by neighboring cells (Salvesen, 2002; Vera *et al.*, 2005; Kuribayashi *et al.*, 2006). Cell death by apoptosis is a part of normal development and maintenance of homeostasis but is also involved in pathological situation associated with sterility. In the testis, apoptosis is such a common programmed event that 75 % of germ cells are reduced by spontaneous apoptosis (Knudson *et al.*, 1995; Kua & Kuida, 2003). In the testis have been described two pathways for cell death mainly, the Fas/FasL system and intrinsic apoptotic pathway. The first, Binding of Fas to its ligand FasL forms a highly stable supramolecular cluster which promotes processing and stabilization of caspase-8 (Francavilla *et al.*, 2000, 2002; D'Alessio *et al.*, 2001; Henkler *et al.*, 2005). Once activated, caspase-8 can activate the effector caspases-3 (Salvesen; Shi, 2002; Codelia *et al.*, 2008), which degrades a large number of cellular proteins that finally kill the cell. The

second, the intrinsic apoptotic pathway is activated by stress, starvation or radiation, and it starts with the assembly of a multimeric complex (apoptosome) involving Apaf-1, procaspase-9 and cytochrome c. Once activated, caspase-9 cleaves and activates caspases-3, ending with the death of the cell.

Extracellular metalloproteases as inducers of germ cell apoptosis: A working model

ADAM10 and ADAM17 are two widely expressed metalloproteases linked to the ectodomain release of not only of ligands, but also receptors such as: Notch, epidermal growth factor (EGFR), the p75 neurotrophin receptor (p75NTR) or c-kit (Blobel, 2005; White *et al.*, 2005; White Laboratory Web Page, 2015). Despite that ADAM1, the first member of the ADAM family, was discovered in the testis, it was not until 5 years later that TACE (the convertase of tumor necrosis factor-alpha) was recognized as a member of the metalloprotease family (Black *et al.*, 1997). Since then, TACE/ADAM17 has been established as a key protease in mammalian development. Several studies have shown that the receptor tyrosine kinase c-kit is a substrate of ADAM17 (Cruz *et al.*, 2004). This receptor (c-kit) is a glycosylated transmembrane protein of 145 kDa, that once bound to its ligand (SCF) initiates a signaling cascade leading to proliferation, differentiation, migration and survival (Bedell & Mahakali Zama, 2004; Matsui *et al.*, 2004; Ronnstrand, 2004). In studies with cultured stem cells derived from mastocytes and umbilical cord endothelial cells, it has been found release of c-kit ectodomain dependent on TACE/ADAM17 activity (Bedell & Mahakali Zama; Matsui *et al.*). Moreover, embryonic cells showed a significant increase in apoptosis after being incubated with PMA, a phorbol ester known to activate PKC, but not in cells expressing a catalytically inactive mutant of TACE/ADAM17 (Cruz *et al.*). This result suggests a strong link between TACE/ADAM17, the processing of c-kit ectodomain and activation of apoptosis. C-kit is expressed in spermatogonia and spermatocyte and bind to its ligand bear by the sustentocyte. Our own data shows that germ cell undergoing apoptosis lack the extracellular domain of c-kit and in vivo PMA induces apoptosis in germ cells of 21-day old rat, which was prevented when a pharmacological inhibitor of ADAM17 was present

at the time of PMA administration (Lizama *et al.*, 2010c). ADAM17, but not ADAM10 is upregulated in germ cell undergoing physiological apoptosis, and pharmacological inhibitors of ADAM17 are able to significantly prevent physiological apoptosis. Overall, these results uncover a new role for ADAM17 as a key physiological regulator of germ cell apoptosis during mammalian spermatogenesis.

External insults that trigger germ cell apoptosis are also related to ADAM17/ADAM10 activation: Interestingly, external insults that induce germ cell apoptosis could also induce activation of ADAM17, suggesting that this enzyme is at the center of different stress events that lead to apoptosis (Lizama *et al.*, 2011b; Urriola-Munoz *et al.*, 2014a, 2014b). An *in vitro* study show that etoposide, an anti-cancer drug that *in vivo* promotes germ cell death, induce apoptosis of two cell lines derived from germ cells (GC-1 and GC-2). Interestingly, etoposide induces upregulation of mRNA and protein levels of ADAM10 and ADAM17. Accordingly, apoptosis could be prevented by a pharmacological inhibitor of ADAM10 and ADAM17 (Lizama *et al.*, 2011a). *In vivo*, etoposide also induces activation of both metalloproteases suggesting similarities in this mechanism (Codelia *et al.*, 2008, 2010; Ortiz *et al.*, 2009). On the contrary, heat stress does not induce ADAM10 or ADAM17 activation, suggesting that not all the insults that induce germ cell apoptosis induce metalloprotease activation.

Recently it has been shown that several environmental endocrine disruptors such as Bisphenol-A (BPA) and nonylphenol (NP) induce germ cell apoptosis (Urriola-Munoz *et al.*, 2014a). BPA and NP xenoestrogens are produced in larger volume worldwide as inducers of polymerization and they can be found in plastics, toys, food cans, cosmetics, etc. (Lagos-Cabre & Moreno, 2012). These are artificial compounds having structural and functional similarity to estrogens, and also have estrogenic i.e. deregulate the hormonal balance of animals and humans, disrupting, estrogen-dependent reproductive (Park *et al.*, 2009). However, xenoestrogens have a low affinity to genomic estrogen receptor (ER α and ER β) compared with estradiol 17 β (Gould *et al.*, 1998), but still may affect the functions and development in animals at low concentrations.

The increase of xenoestrogens in the environment has raised concerns about the importance of these on human health, because they have been detected in human samples such as serum, urine, amniotic fluid, breast milk, semen, etc. (Inoue *et al.*, 2002; Calafat *et al.*, 2005; Swan *et al.*, 2005; Main *et al.*, 2006). And they have been linked to a decrease in anogenital distance in sperm quality and fertility of man (Swan *et al.*).

There is a large volume of information regarding the disruption generated by BPA or NP in spermatogenesis using animal studies (Lagos-Cabre & Moreno). A decrease in the weight of testis, epididymis and sperm count has been observed in rats treated with up to 100 mg / kg / day of NF (Chitra *et al.*, 2002). In addition, it decreases count and motility of sperm in adulthood when male rats are treated from 100-1600 mg / kg / day BFA during the neonatal period (Salian *et al.*, 2009).

The decrease in number of sperm count in rats treated with xenoestrogens relates to an increase in germ cell apoptosis because of an activation of caspase-3 in germ and Leydig cells, increased levels of Fas in germ cells and FasL in Sertoli cells and increased TUNEL positive cells (Watanabe *et al.*, 2004; Tomasini *et al.*, 2008; Hu *et al.*, 2013). With respect to humans, there has been a high correlation between levels of BPA in urine and sperm abnormalities (motility, viability, count and sperm concentration and DNA fragmentation), suggesting that BPA and NP induce apoptosis of germ cells in humans (Meeker & Ferguson, 2011). Therefore, given the ubiquitous distribution of these compounds in plastic products used by humans, it is important to clarify its mechanism of action and also to generate new pharmacological tools for infertility.

Recently it has been shown that the treatment with BPA or NP induces activation of ADAM17 but not ADAM10 in 21-days old rat. Accordingly, the apoptosis (mainly spermatocytes) was prevented when rats were previously treated with an ADAM17 but not ADAM10 pharmacological inhibitor (Urriola-Munoz *et al.*, 2014a). *In vitro*, BPA and NP promote the shedding of TNF- α in cultured sustentocytes, suggesting that effectively they induce activation

of ADAM17. Interestingly, BPA and NP induce shedding of neuregulin (another substrate of ADAM17) at similar concentration found in human fluids, suggesting that it might be possible that in vivo these compounds could affect this enzyme.

Final considerations

Apoptosis during spermatogenesis seems to be important to control the proper progression of germ cell development and the ration between germ cells to sustentocyte. However, to understand the physiological basis of this process help us understand its role in different pathological condition that may affect system beyond reproductive organs. The available data show that under physiological conditions, germ cell death could be considered as a bona fide apoptosis process, which seem to be regulated by ADAM17. Interestingly, despite the wide distribution of this enzyme, this is the only reported case where it is involved in apoptosis.

Other reports have shown its involvement in cell signaling, differentiation, inflammation or cell degeneration. Thus, it seems that male germ cells are unique in the mechanisms used to regulate apoptosis.

These data raise several questions such as: Which is/are the physiological substrate(s) shedding by ADAM17 during germ cell apoptosis? How ADAM17 is activated under physiological conditions? How ADAM17 is activated under pathological conditions? Is there any physiological role for ADAM17 in mammalian spermatogenesis?

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RESUMEN: La apoptosis durante la espermatogénesis en mamíferos, es importante para controlar la salida de espermatozoides y para eliminar las células dañados no deseadas. Una desregulación en la tasa de apoptosis tiene consecuencias deletéreas y conduce a la baja producción espermática. La apoptosis en la espermatogénesis se ha estudiado ampliamente, pero el mecanismo por el que se induce en condiciones fisiológicas o patológicas no se esta clara. Recientemente hemos identificado a la metaloproteínasa ADAM17 (TACE) como una inductora de la apoptosis fisiológica en células germinales. La enzima ADAM17 está ampliamente distribuida en muchos tejidos, su función principal es liberar sus sustratos, lo que conduce a la activación de diferentes vías de señalización de acuerdo con el contexto celular. Sin embargo, en la espermatogénesis, ADAM17 está involucrada en el control o inducción de la apoptosis de las células germinales, esto parece ser único y puede reflejar la complejidad de la espermatogénesis. En esta revisión expone la evidencia de cómo esta enzima se muestra implicada en el control de la apoptosis de células germinales durante el estado fisiológico y en diferentes insultos tales como el estrés por calor, el agente que daña el ADN o los disruptores endocrinos.

PALABRAS CLAVE: ADAM17; La apoptosis; Célula germinal masculina.

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Correspondence to:
Ricardo D. Moreno
Departamento de Fisiología
Facultad de Ciencias Biológicas
Pontificia Universidad Católica de Chile
Alameda 340
Santiago
CHILE

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Email: rmoreno@bio.puc.cl