Advances in Life Science and Technology
ISSN 2224-7181 (Paper) ISSN 2225-062X (Online)
Vol. 17, 2014

Seminal fluid; the Natural Guard of Seminal DNA

"Mini-Review"

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The ability of foreign DNA to be introduced into the sperm cells faces many problems. These problems have been emerged from the existence of some inhibitory factors available in the seminal fluid. Add to that, other several factors are involved in this internalization. The purpose of this review is to evaluate the protective or interactive roles of these seminal fluid proteins in the process of foreign DNA internalization into the head of the sperm.

The ability of foreign DNA to be internalized inside sperm cell is become obvious before more than four decades (Brackett *et al*., 1971). But, the mechanism by which this foreign DNA has the ability to do so is still under debate. However, several papers described certain factors involved in this process. Thus, in order to describe the mechanism of sperm transfection by exogenous DNA, it is necessary to understand the following natural factors that are playing main roles in this scene and they include; inhibitory factor I (IF1), seminal fluid DNase, DNA binding proteins (DBPs), CD4, major histoincompatibility complex class II (MHCII), topoisomerase type II (TOPO II) and reverse transcriptase (RT). They can be further classified into three main groups.

The First group is represented by two factors found in seminal plasma; IF1 and DNase, this group is responsible about inhibiting the internalization process of exogenous DNA. The fact which refers to the existence of one or more factors in seminal plasma that able to block sperm permeability must be taken into account. This means, extensive washing steps of ejaculate to remove seminal plasma is necessary and should be made before incubating sperm with exogenous DNA. Lauria and Gandolfi reviewed that seminal fluid inhibitors have two ways of inhibition to exogenous DNA, either directly or indirectly (Lauria and Gandolfi, 1993). These seminal plasma inhibitory factors may prevent transfection of intact sperm by foreign DNA (Camaioni *et al.*, 1992). Gandolfi showed that there is a consensus on the experiments made on seminal fluid of the ejaculated spermatozoa of mammals in the impermeability of sperm cell to the aggression of foreign DNA as long as seminal plasma is not removed (Gandolfi, 2000). Thus, seminal fluid prevents any foreign DNA from binding with its receptor on the sperm cell.

The IF-1 showed "a powerful inhibitory effect" on the process of DNA uptake in sperm cells. The 30- to 35KDa DNA binding proteins appeared to be the specific target through which the inhibition of foreign DNA uptake is taken place. In the presence of this inhibitory factor, exogenous DNA losses its ability to bind with the sperm surface. Consequently, the process of interaction of exogenous DNA with sperm cells is strictly mediated by several specific factors controlling sperm-DNA interaction which represent a natural barrier in seminal fluid against the entry of any foreign DNA (Zani *et al.*, 1995; Lavitrano *et al.*, 2006). These evidences are clearly indicated the necessity to remove seminal plasma before SMGT technique is being done (Sato, 2005).

Another inhibitory factor found in seminal fluid that should be taken into account is seminal fluid DNase. A study revealed that even after washing, sperm contain DN*ase*, which usually degrades the foreign DNA. This enzyme is abundant in seminal plasma and variable amounts of the enzymes remain bound to sperm, this in turn deciphers why the experiment of DNA-sperm interaction was failed and why foreign DNA was fragmented. A possible interpretation of this phenomenon is that DNase is a tool used by seminal fluid to protect sperm from contamination by any intruding DNA during its journey from the epididymis to the oocyte (Houdebine, 2003; Baccetti and Spadafora, 2000).

The second group is represented by DBPs, CD4 and MHCII – and it is responsible about assisting the uptake and internalization process of exogenous DNA. Before exploring this group altogether, it is necessary to put an attention on DBPs considering them as a "receptors" which act as substrates for the exogenous DNA before its possible penetration into the sperm cell (Gandolfi, 2000).

In the absence of seminal fluid, the medium that containing several inhibitory factors,the DBPs may be free to interact with DNA to form a complex capable of transporting inside the sperm cell. For this reason, Lavitrano's group emphasized once and again the crucial necessity to remove seminal fluid after ejaculation as soon as possible before undergoing SMGT (Lavitrano *et al.*, 1997; 2006).

According to molecular weight, three DBPs have been isolated; they include (50 KDa), (30-35 KDa), and below (20 KDa) DBPs. The middle (in molecular weight) DBP gained particular attention because its electrophoretic mobility is conserved among many species, in addition, it was believed that this type is the only DBP accessible to foreign DNA in the intact sperm acting as a physiological substrate (Zani *et al.*, 1995).

Other supporting factors for DNA uptake and internalization are discovered by Lavitrano's group (1997). They demonstrated that CD4 and MHC class II molecules play significant roles in the process of foreign DNA uptake and internalization: though not present in mature sperm cells, MHC class II expression appears to be required during spermatogenesis to produce sperm cells capable of taking up foreign DNA, while CD4 molecules, are shown to be found on sperm head of wild-type animals, and mediate the nuclear internalization of sperm- bound DNA (Lavitrano *et al.*, 1997). Some researchers considered this discovery as a first steps toward understanding foreign DNA internalization after its uptake (Gandolfi, 1998). Thus, CD4 as antigen, participate in a speculated model in which foreign DNA bind to one of DBPs molecules (the30-35 KDa) that present on the sperm surface in the absence of seminal fluid.

After binding, nuclear internalization of foreign DNA takes place within minutes by CD4 molecules that present on the sperm membrane. The resulting complex, The DNA-DBP-CD4, leaves the sperm head surface and penetrates nuclear pores to access the nuclear matrix where DNA is dissociated from the DBP-CD4 complex. But some papers showed a dramatic conversion in the metabolically inert sperm nucleus since an endonuclease activity is triggered just after the entry of foreign DNA. Then it will be consequently fragmented in a typical manner. This metabolically active process is similar to apoptosis which destroys both the DNA and the sperm cell (Maione *et al.*, 1997; Spadafora, 1998; Gandolfi, 2000). However, there is a possibility for this DNA to be integrated into the genome after its cleavage by endonucleases (Sato, 2005). This conclusion leads us to third group.

The third group is represented by topoisomerase Type II and reverse transcriptase, and it's may be correlated directly or indirectly with the integration process. It has been shown that foreign sequences are inserted in a unique integration site in the sperm genome (Magnano *et al.*, 1998). Studies in mouse suggested, that plasmid DNA internalized in ejaculated spermatozoa associates with the nuclear scaffold, is extensively rearranged, and undergoes nonhomologous recombination with genomic DNA. This may indicate a direct role played by topoisomerase type II in this process (Zoraqi and Spadafora, 1997; Wolf *et al.*, 2000). The presence of a topoisomerase II cognate site suggests that DNA double-strand breaks have been generated as a step in this process (Spadafora, 1998).

The first time discovery for the correlation of reverse transcriptase with this event has been came with the evidence provided by Giordano *et al.* (2000). By such activity, the exogenous RNA molecules were able to be retrotranscribed to cDNA copies (Giordano *et al.*, 2000). This activity is endogenous that is triggered by the exogenous RNA in sperm cell. Consequently, this gives another hint for retrotransposon/retroviral machinery to involve itself in SMGT at the molecular level (Smith and Spadafora, 2005).

Irrespective of previously mentioned mechanisms, the integration frequency is relatively low, and the mechanism is still not well known (Niu and Liang, 2008). However, despite the fact that the sperm genome is strictly packaged in an stable structure with a very little biochemical accessibility (Shaman and Ward, 2006) but this doesn't mean that this genome is surrounded entirely by such structure since there are some exposed regions of this genome which may serve as potential sites for exogenous DNA integration (Wall, 1999).

In conclusion, the story of the exact role of seminal fluid in interacting with the exogenous DNA is still under continuous controversy. Moreover, there is a paucity of information mentioned about these factors. Therefore, much more investigations have to be performed in order to elucidate the molecular levels of seminal proteins interventions with outer environments including the exogenous DNA and the inner environment that represented by the sperm cells molecules.

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