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RESEARCH ARTICLE

SPERM MEDIATED GENE TRANSFER (SMGT) - A NOVEL APPROACH TO PRODUCE TRANGENIC ANIMALS

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ABSTRACT

As the conventional methods of transgenic animals are affected from low efficiency and high cost, the use of sperm as vector for gene transfer is appeared to be interesting and promising. Gene delivery to offspring by this method is called as sperm-mediated gene transfer (SMGT). The SMGT appears to be simple, efficient, and relatively inexpensive methods in modifying animals and the genome of animals. Several factors determine the success of SMGT include, the donors of spermatozoa, incubation media, exogenous DNA size and type and the assisted reproductive technique used. SMGT has been used more or less successfully in the production of transgenic embryos and animals in a large number of species.

INTRODUCTION

Transgenic animals have become valuable tools for both research and applied purposes. The conventional methods of transgenic animal productions are suffered from poor efficiency, requirement of specialized laboratory techniques, need of high-skill to manipulate embryo at early stages of development and safety issues raised by use of viral vectors. Over the recent years, a variety of transgenic approaches have been assessed and developed in the pursuit of a more efficient and easier method. Among them, the use of sperm cell as the vector for gene transfer is much interesting and promising (Lavitrano *et al*, 1989; Zani *et al*,1995). Gene delivery to offspring by this method is now called sperm-mediated gene transfer (SMGT). This technique has the potential to become the most efficient and cost effective method to generate transgenic animals, which will significantly increase application in biomedical research and in commercial production. Although transgenic animals have been obtained using SMGT, its efficiency is poor, mainly due to the spermatozoa's lesser uptake of exogenous DNA, thereby reducing the number of fertilized oocytes with transfected spermatozoa (Anzar and Buhr, 2006).

The possibility of binding exogenous DNA by mammalian spermatozoa was first reported 42 years ago (Brackett *et al*, 1971) showed rabbit sperm could take foreign DNA. However, this information was disregarded until Lavitrano *et al* (1989) demonstrated the transfer of exogenous DNA by mouse epididymal sperm to the oocyte resulting in transgenic offsprings with 30% efficiency. After that several studies have reported various success stories in different animal species like chicken (Nakanishi and Iritani, 1993), mouse (Maione *et al*, 1998), Xenopus (Jonak, 2000), zebrafish (Khoo, 2000), pig (Lavitrano *et al*, 2002), bovine (Anzar and Buhr, 2006) and goat (wang *et al*, 2011).

The mechanism of gene delivery to spermatozoa

Although the DNA uptake by spermatozoa is well documented (Lavitrano *et al*,1989; Parrington *et al*, 2011), both mechanism and its regulation are still poorly understood. The exogenous DNAs bind to the sperm head in the acrosomal region and in the proximity of equatorial area. Once bound to the cell membrane, DNA molecules are taken up (Francolini *et al*, 1993). MHC class II molecules and the antigen CD4, present in the posterior region of the sperm head are responsible for the high degree of affinity of sperm to DNA. The DNA becomes tightly associated to nuclear proteins when enter in to the nucleus (Magnano *et al*, 1998; McCarthy *et al*, 2000). Then sperm endonucleases cleave it and the DNA was finally integrated in to the genome (Maione *et al*, 1997). Reports suggest that the mechanism of DNA integration controlled by a complex net of factors, secreted by and linked to the sperm. Mouse seminal plasma containing a DNAse and diverse exogenous DNA binding proteins are found to be responsible for inhibitory activity to exogenous DNA sequestration (Carballada and Esponda, 2001). Although the seminal fluid strongly antagonizes foreign DNA binding, under normal conditions it act as strong protection of sperm cells against foreign DNA (Celebi *et al*, 2003). A glycoprotein, inhibitory factor-1 (IF-1) abundant in the seminal plasma of mammals and on the surface of spermatozoa of invertebrates exerts an inhibitory effect on binding of exogenous DNA to sperm membrane (Arezzo, 1989). Therefore, IF-1 has an important natural role, acting as a barrier and protecting sperm against the entry of undesirable exogenous molecules, which could compromise the sperm integrity and the genetic identity of the future progeny (Spadafora, 1998; Spadafora *et al*, 2002). This account for the observation that the seminal plasma must removed as a part of SMGT protocol.

Methods used for uptake of foreign DNA by spermatozoa

SMGT can be broadly classified into two distinct categories

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, 'Auto' and 'Augmented' uptake of naked DNA molecules. The first category includes incubation of DNA with spermatozoa. The augmented uptake contains different ways to increase the capability of spermatozoa to capture exogenous DNA such as, utilization of liposome (Bachiller *et al*,1991), electroporation (Gagne *et al*,1991), virus mediated transfection (Nagano *et al*, 2000), Linker (receptor) based method (Wu and Wu, 1987), combination of restriction enzyme-mediated integration (REMI) with SMGT (Kroll and Amaya, 1996), as well as combination of intracytoplasmic sperm injection (ICSI) with sperm/DNA interaction (Perry *et al*,1999).

DNA incubation

Incubation of foreign DNA with spermatozoa, termed "DNA incubation", is the direct method reported by Brackett et al, (1971). This approach suspends the seminal plasma-free sperm cells in an appropriate medium containing foreign DNA molecules. Then the resultant sperms carrying foreign DNA are used to fertilize eggs by *in vitro* fertilization (IVF) or AI. A number of studies further showed that a variety of molecules or factors contributed to the interaction of exogenous DNA and spermatozoa. To date, a variety of transgenic mammals, such as mice, rats, rabbits, pigs, goats and cattle, have been produced using the DNA incubation method. Researchers reported that the overall efficiency of SGMT was reported to be higher when ejaculated spermatozoa were used, compared to epididymal spermatozoa. While development of transgenic embryos was a DNA dose-dependent effect in which high DNA doses arrested the embryonic development (Scia-manna *et al*, 2000). Sperm from different species exhibited a wide variation in DNA binding efficiency ranging from 0.3 to 78% suggesting a species specific mechanism (Camaioni *et al*, 1992). Breeds of goat are also reported to influence rate of DNA uptake which ranged from 3.08 to 73.39% (Wang *et al*, 2011). In bovine, neither bull nor DNA concentration could influence the spontaneous DNA uptake. However, frozen-thawed bovine sperm showed a better DNA binding efficiency than fresh spermatozoa (Canovas *et al*, 2010). In pig, more than 50% of the sperm is reported to bind to DNA after co-incubation (García-Vázquez *et al*, 2011).

Liposomes mediated gene transfer

Liposomes are small artificial vesicles of spherical shape that have been used for a long time as drug carriers and loaded with a great variety of molecules, such as small drug molecules, proteins, nucleotides and even plasmids into living cells (Gregoriadis and Allison, 1980). Bachiller *et al* (1991) used liposomes for the sperm mediated gene transfer and obtained satisfactory efficiency of DNA transfer with no reduction in the fertilization rate. However, authors couldn't produce transgenic mice using this method. Other studies showed that liposome treated exogenous DNA can be captured efficiently by sperm cells and transmitted to next generation (Wang *et al*, 2003; He *et al*, 2006). However, a very little is known about the mechanistic aspects of liposome-sperm cell interactions. Furthermore, the existence of several different types of liposome makes it difficult to make general predictions as to the likelihood of success, in the absence of specific empirical studies. The selection of the leptosomes also affects the DNA integration in to the genome of progeny (Yonezawa *et al*, 2001)

Linker (receptor) based method

In 1987, one study reported receptor mediated gene transfer using polycation-conjugated asialoglycoprotein (Wu and Wu, 1987). DNA can bind to polycations in a strong but non - covalent manner forming soluble complexes. DNA coupled with antibodies or antibody-fragments offers the ability to internalize the complexes via receptor-mediated endocytosis (Varga *et al*, 2000). Qian *et al* (2001) firstly reported the use of monoclonal antibody (mAbC) as linker for SMGT to generate transgenic mice and chicken. Monoclonal antibody (mAbC) was used as linker to transfer exogenous DNA to sperm and after artificial fertilization, viable pigs were produced (Chang *et al*, 2002). They also demonstrated that the foreign DNA could be specifically bound to the sperm cell surface via the linker protein (mAbC) through ionic interaction. These data indicate that linker-based SMGT can be used to generate transgenic animals efficiently in many species, especially in the farm livestock.

Virus mediated method

Farre *et al* (1999) used adeno viral vector to introduce foreign DNA in to the sperm and demonstrated the presence of lac Z gene in the head of spermatozoon. The transfected sperm produced lac Z expression in 21.7% embryos. Researchers failed to produce germ cell transduction when spermatogenic cells and mature sperms were exposed to high titre of adenovirus and subsequently subjected to *in vitro* fertilization (Hall *et al*, 2000; Gordon, 2003). The use of lentiviral or adeno associated viral (AAV) vectors, both of which are recognized as promising vector for gene therapy (Lotze *et al*, 2002) appears to be of interest in SMGT. Later Couto *et al* (2004) demonstrated that the direct exposure of murine spermatozoa to very high concentrations of AAV failed to transduce germ cell. If these vectors were able to transduce sperm, exposure of sperms to large amount of vectors prior to fertilization would be expected to lead to early integration. However, till yet it is not proved why mature sperm cannot be infected by viral vectors.

Electroporation

Electroporation (EP) has been used for both obtaining stable transformants in eukaryote cell lines and introducing plasmids into bacteria. Gagné *et al* (1991) reported electroporation mediated DNA uptake by bovine spermatozoa *in vitro*. Their study demonstrated that foreign DNA could be stably captured by spermatozoa following EP treatment to be carried into oocytes during fertilization. Patil and Khoo (1996) also performed electroporation on zebrafish and showed that electroporated sperm cells took up more DNA molecules than those not subjected to the electrical field. Tsai *et al* (1997) reported the transgene was transmitted to the first generation in 65% of cases and integrated into the genome of some larvae of mollusk, *Haliotis divorsicolor suportexta*. The homologous recombination is also possible using SMGT in cattle (Rieth *et al*, 2000). Indeed, transgenic embryos were obtained after electroporating sperm with DNA constructs carrying a reporter gene and a highly repetitive, Alu-like repeat known to favor transgenesis by homologous recombination.

Restriction enzyme-mediated integration

In 1996, Kroll and Amaya introduced linearized plasmid DNA into the nuclei of *Xenopus* sp. sperm by using restriction

enzyme-mediated integration (REMI) to decondense the genomic DNA. The transgenic nuclei were transferred into unfertilized eggs by microinjection of sperm nuclei, and 36% of the *Xenopus* sp. offspring obtained were found to be transgenic, compared to 19% when the restriction enzyme was omitted from the reaction. The REMI technique was also applied successfully to cattle (Shemesh *et al*, 2000) in which it was combined with lipofection to integrate the transgene into the genomic DNA of the sperm before *in vitro* fertilization.

Intracytoplasmic sperm injection after sperm-DNA interactions

Other techniques involving intracytoplasmic sperm injection (ICSI) have successfully developed (Wakayama and Yanagimachi, 1998). These methods overcome the problem associated with killing spermatozoa as a result of applying different treatments to help the exogenous DNA cross the natural sperm barrier during transfection. Thus, spermatozoa is subjected to Triton X-100 treatment, repeated freeze-thaw cycles, or freeze drying cycles before incubation with exogenous DNA (Wakayama and Yanagimachi, 1998; Perry *et al*,1999) can generate transgenic offspring. Rhesus macaque embryos expressing a transgene were produced by this approach.

CONCLUSION

In conclusion, SMGT appears to be simple, efficient, and relatively inexpensive methods in modifying animals and the genome of animals. Several factors determine the success of SMGT include, the donors of spermatozoa, incubation media, exogenous DNA size and type and the assisted reproductive technique used. However, its underlying molecular basis is generally neglected and the inconsistencies concerning the reproducibility associated with this method remain unsolved.

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