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Gene Therapeutic Enhancement of Animal Health and Performance: Review

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Abstract: Gene therapy is a new modality with the potential for treating or preventing a variety of inherited or acquired diseases. The last 10 years have seen substantial progress in the development of gene therapy. New viral and non viral vectors and applications are being developed at a rapid pace. The progresses in vector targeted gene therapy, improves both gene therapy safety and fulfils its potentials as a therapeutic modality. Significant progress has been made in the continued development of viral systems including retrovirus, adenovirus, adeno associated, herpes virus as well as the exploitation of novel tools such as plasmid DNA herpes virus based systems. New vaccines using DNA vectors are used to long lasting immune response. Also the technology is important to administer growth hormone releasing hormone through plasmid DNA. The application therefore can be extended to enhance the economic efficiency of animal food production and over all animal health and performances.

Key words: Animal · Gene therapy · Vaccine · Vectors

INTRODUCTION

Gene therapy is a method of prevention or treatment specifically used to treat patients who are suffering from diseases due to defective genes. Essentially, the treatment involves researchers replacing the defective or faulty genes with a normal functioning gene. It involves detection of gene, determination of its role, cloning and introducing the gene by proper way. This is either germ line gene therapy (done in germ cells) or somatic gene therapy (done in somatic cells) [1, 2].

Gene therapy is often aimed at achieving a long lasting physiologically matched expression of the gene, without activating the immune system. The aim is even to integrate the genetic materials into the chromosome. Gene therapy promises to revolutionize agriculture as well as medicine. We are nearing the end of the first decade of gene therapy and important recent developments were made. The early results on the clinical efficiency of gene therapies were disappointing, largely because the available gene transfer vectors provoked to be inadequate. Great progress was made in selecting and improving vectors and subsequently first positive results were reported [3].

The use of molecular and cellular tools to genetically modify and improve food supply is playing a leading role in the continuing concern to produce more food and of a better quality for the increasingly demanding world population. Genetically manipulated animals generated by transgenic and gene targeting (knockout) technology contributed tremendously to improve productive efficiency of animal agriculture, increase milk and meat production [4, 5]. Many of the approaches supplement or enhance breeding, address environmental concerns or stabilize food production [6].

Studies indicate that often the public perceives no consumers benefits from farmers' use of recombinant proteins or transgenic animals [7]. Investigators focused on developing a new type of technology in agriculture field, approaches of gene therapies rather than transgenesis for improving growth and lactation in farm animals by enhancement of endogenous animal performances or for vaccine production [6]. In general during the last few years substantial progress in the development and application of gene therapy have seen but many problems remain to be resolved before delivery of genes for improvement of animal health and performances. The achievement of these goals has broad

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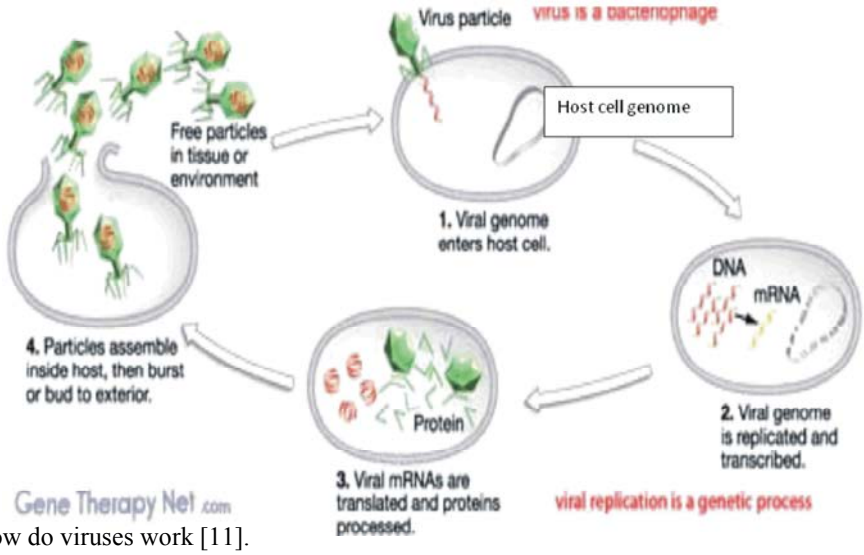

Fig. 1: The way how do viruses work [11].

ramifications and finally will enhance the economic efficiency of animal food production [8]. This paper is aimed to review different gene therapeutic enhancement benefit and assess types of gene therapeutic vectors.

Overall Review of Gene Therapeutic Enhancement: In gene therapy, the genetic material must reach the right cells, as well as generate the desired degree of activity. The very complex and challenging problem of 'gene delivery' is how to get the new or replacement genes into the desired tissues. A means of efficiently delivering working copies of the gene to these cells is using vectors. When choosing vector for gene therapy, there are some fundamental differences between vectors available in terms of capacity of transgene cassette, duration of trans-gene expression, immunogenicity and ability to target quiescent or dividing cells and extra chromosome or genomic integration of transgene as well as basic safety issues [9].

Viral Vectors: All viruses attack their hosts and introduce their genetic material into the host cell as part replication cycle. This genetic material contains basic instructions of how to produce more copies of these viruses (Figure 1). The host cell will carry out these instructions and produce additional copies of virus, leading to more and more cells becoming infected. Some type of viruses actually physically inserts their genes into the host's genome. This incorporates the genes of that virus among the genes of the host cell for the life span of that cell. Recombinant DNA technology has made it possible to insert and express hetrologous genes in a variety of different viruses [10].

Viruses are vehicles that efficiently transfer their genes into host cells. This ability made them desirable for engineering virus vector systems for the delivery of therapeutic genes. The viral vectors currently used in research are based on RNA and DNA viruses processing genomic structures and host ranges. Particularly viruses were selected as gene delivery vehicles because of their capacities to carry foreign genes associated with efficient gene expression [11]. These are the major reasons why viral vectors derived from retroviruses, adenoviruses, adeno-associated viruses and herpes viruses are employed in more than 70% of clinical gene therapy trials worldwide [11].

Retrovirus Vectors: Retroviruses are a group of RNA containing viruses characterized by the employment of the unique molecular mechanisms which is an efficient transfer system. By the reverse transcriptase enzyme activity the viral RNA genome to be transcribe into double stranded DNA that stable integration into host DNA. The advantage of retroviral vectors are the stable integration into the host genome, generation of titers that allow efficient gene transfer into a broad variety of target cells and the ability to carry foreign gene up to eighty kb [9]. Vectors based on lentiviruses such human immune deficiency virus have the ability a variety of post mitotic tissues as heart, muscle or brain, but biosafety remains a major in production of such vectors [12].

Despite the above problem, retroviruses are the system of choice for some animal gene therapy applications like somatic gene therapy. Somatic gene therapy consists in stable expression of a transgene product from an implanted group of cells that could be eventually removed if desired. The vectors used in these cases are retroviruses because of their property of integrating into the transduced cells genome and express the transgene for the sequential generations in the cell-line [13]. Investigators developed stable transfections of porcine myoblasts and fibroblasts isolated from muscle of young pigs were transduced with vesicular stomatitis virus glycoprotein G pseudotyped retrovirus and resulted in efficiencies of 1: 1.2 for myoblasts and 1: 1.1 for fibroblasts. As this experiment suggested that cell-mediated gene transfer is possible in porcine muscle and the technology is useful as an approach for promoting muscle growth in pigs [14].

As a step further this strategy, also possible to deliver long-term growth hormone (GH) to swine [15]. Hormones stimulating growth and lactation are the obvious targets of animal gene therapy. Administration of GH to farm animals has been used to improve muscling and milk production, while improving feed efficiency. Administration of porcine GH to growing pig increases average daily weight gain by 10-20%, improves feed efficiency by 10- 30%, decreases lipid accretion rates to 70% and stimulates protein deposition (muscle growth) to 60% [16]. The porcine GH gene was constructed inside a bicistronic retroviral vector transfected into fibroblasts further encapsulated with immunoprotective microcapsules [4].

Adenovirus Vectors: Adenovirus capsids are ecosehedral particles comprised of three major component of proteins the hexon, pentone base and fiber [17]. Adenoviruses have been powerful tool in the development of experimental gene therapy for autoimmunity. Their ability to infect dividing and non dividing cells with high efficiency and the capacity to produce high titer adenoviruses stocks have greatly aided their applications. Adenovirus two and adenovirus five are the two adenoviral serotypes most commonly used as vectors for gene therapy. There are numerous examples of the successful application of adenoviruses in gene therapy of animal model of virus autoimmune diseases including diabetes [18], multiple sclerosis, rheumatoid arteritis, systemic lupus erythrthematosus [19], autoimmune myocarditis. Furthermore, adenovirus vectors can infect a wide range of cells [11]. Recently, targeted viral vectors localize gene transfer to specific cell types, thus reducing immunogenicity and toxicity, increase safety and enable the system with administration of these vectors for multiple indications including cancer, cardiovascular diseases and inflammatory diseases. Even if the adenoviruses episomal state within the host cells allows only transient expression, some adenovirus gene provokes inflammatory reactions and toxicity that limits repeated admistration [17].

Adenoviral vectors are also an alternative to the introduction of a manipulated gene into the spermatozoa as gene therapy for the next generation. For instance exposure of boar spermatozoa to adenovirus DNA bearing Escherichia coli lacZ gene transfer the gene to the head of the spermatozoa and to offspring. Treatment did not affect either viability or acrosomal integrity of boar sperm. Approximately 22% of cell embryos obtained after in vitro fertilization with adenovirus-exposed sperm expressed the LacZ product. Also, 7% of the piglets obtained after artificial insemination with adenovirus-exposed spermatozoa showed the presence of the LacZ mRNA in all of the tissues tested. The offspring obtained after mating of the two positive animals did not show LacZ gene presence. Their results indicate that adenovirus could be a feasible mechanism for the delivery of DNA into spermatozoa, even though the transfer of the transgene gives the impression to be limited to the first generation [20].

Adeno-Associated Virus Vector: Adeno-associated virus (AAV) is a small less than 5 kb, single-stranded DNA non-enveloped parvovirus. The natural route of infection of AAV is the upper respiratory tract. For productive infection to occur, AAV requires co-infection with Ad which allows the viral genome to replicate episomally and leads to synthesis of AAV proteins. The AAV requires an adenovirus or a herpes virus for viral replication. No pathology was linked to this virus [17].

As a gene therapy vehicle the AAV has important characteristics: high level of infection in different cell types, including post-mitotic cells, long-term expression of the transgene for at least up to 2 years [21], low immunogenicity and could integrate in a specific site on chromosome 19 (property often lost in AAV gene therapy construct). Unfortunately, the recombinant AAV production requires super infection with an adeno-virus

that results in low-quality viral stocks. Another potential rate-limiting aspect is the relatively small packaging capacity, less than 5kb [22]. Based on impressive success in delivering genes by this method in rodents and primates, many clinical trials using AAV are ongoing for a variety of human inherited diseases [1]. In contrast, this type of vector is relatively unused for animal improvement purposes. Studies using rAAV *in vitro* indicated that both dividing and non dividing cells are transduced and these observations are further substantiated by *in vivo* delivery of the virus, which demonstrates efficient transduction of neurons [23], muscle liver and air ways [24]. Indeed, it has been shown that a single injection of virus in skeletal muscle, a tissue considered to be mainly terminally differentiated, results in expression of reporter genes for more than a year in immunocompetent mice [25].

Herpes Simplex Virus Vector: Herpes simplex virus (HSV) is a neurotrophic DNA virus with some favorable characteristics for use in gene therapy applications. The genome of HSV is 150 kb long and encompasses more than 80 viral genes. A large part of the genome is dispensable and can be replaced with foreign DNA without significant effect on viral growth. The huge transgene capacity of HSV permits the introduction of multiple genes along with components for transcriptional regulation. HSV infects cells through a complex process that involves attachment to cells initially through interaction of viral glycoproteins and glycosaminoglycan moieties of cell surface proteoglycans. Secondary interactions then occur between viral glycoprotein D and one of two cell surface receptors termed HSV entry mediator A and C, which leads to fusion of the viral envelope and the cell membrane with consequent release of the virion contents into the cell [13].

The natural life cycle of the virus involves infection of epithelial cells in which it undergoes lytic replication. The released virions can then infect sensory neurones. Within the neurones, the DNA core is transported retrogradely and enters the nucleus. Lytic infection continuous and the virus enter a latent phase in which the viral genome exists as a stable episomal element within neuronal nuclei for the lifetime of the host without disturbing normal cell function [26]. During latent phase, gene expression is limited to a small region which encodes latency-associated transcripts (LATs) under the control of two adjacent promoters LATP1 and LATP2. Replication-incompetent vectors in which these genes are deleted in isolation or combination have been produced and are grown efficiently in cell lines that complement for the absence of the deleted gene [27].

Naked DNA: Plasmid DNA represents the most basic form of DNA whose production is simple and cheap. Unlike viruses, plasmids are non immunogenic, do not encode accessory proteins, they have no innate ability to enter cells, localize to the nucleus or incorporate into the genome. Delivery of plasmid DNA to cells is generally dependent on physical methods, which enable it to penetrate the cell membrane. Having entered a cell, plasmid DNA that is not degraded manages to enter the nucleus and persists as extra chromosomal DNA from which mRNA can be synthesized. Subsequent expression in most cells is transient, continuing for up to a week due to the fact that DNA is lost during cell division or is degraded. Thus, injection of naked DNA into joints achieves low-level transient gene expression [28]. Skeletal muscle is the notable exception to this rule. The organ of choice for plasmid delivery is the skeletal muscle, but skin, some tumors and immune cells are successfully transfected using naked DNA [29].

For farm animals, skeletal muscle possesses properties that make it an attractive target organ for gene therapy. It offers an easily accessible site for injection of DNA and the post-mitotic nature and longevity of muscle fibers permit sustained expression of genes that are delivered. Studies to date suggest that when plasmids are injected into skeletal muscle they persist as an episome and are not integrated into host chromosomal DNA [30] even though expression was reported to persist for up to 19 months [31]. The transfer efficiency of plasmid was reported to be superior to adenovirus and retrovirus in rodent muscle and expression of recombinant protein from intramuscular injection of plasmid were also reported in primates and humans [32].

Comparative with viruses, the non viral techniques for gene transfer *in vivo,* the direct injection of plasmid DNA is simple to use, easy to produce on large scale, inexpensive and safe, as it lacks specific immune response. In addition to expression of reporter enzymes, skeletal muscle is now used as a bioreactor to express therapeutic proteins having either a local or systemic effect [13]. This methodology was limited by the relatively low expression levels due to inefficient DNA uptake into muscle fibers to ensure systemic physiological levels of secreted proteins [8, 33]. Several approaches were

developed to enhance the efficiency of gene transfer via naked DNA including gene gun or electroporation. Electroporation was previously used in un-anesthetized humans to transfect tumor cells after injection of plasmid DNA [34]. The electrotransfer method was first used experimentally in rodents and other small animals [35] and it was found to be effective with expression levels at 40-100 folds over injection alone bringing the range of expression to physiological realm. Different polymers that weakly interact with DNA such as PVP were shown to improve the efficiency of gene transfer [36].

Plasmid DNA for Vaccine Production: Non viral vectors are particularly suitable for creating vaccines, as parts of the viral antigens can be expressed. The generated immune responses are sufficient to protect animals from a wide variety of live infectious agents, leading to the creation of a new class of therapeutic agents, the DNA vaccines. Different studies in mice showed that DNA immunization can induce neutralizing antibodies and cytotoxic T-cells against several viral pathogens, including rabies virus [37], herpes simplex, murine cytomegalovirus or papilloma virus [38]. In porcine, investigators tested pseudorabies virus based on surface glycoproteins B, C and D important antigens implicated in protective immunity against pseudorabies virus infection [11]. Also naked DNA vaccines were developed as candidates for foot-and-mouth disease (FMD) [39]. For example the immune response elicited by pWRMHX in swine indicates that the plasmid encoding the replicating genome stimulated stronger immune responses were partially protected from a highly virulent FMD challenge. Using this methodology, recombinant swinepox virus vaccines expressing pseudorabies virus antigens were developed and shown to provide protection against challenge [40].

Naked DNA for Growth Enhancement: Porcine growth hormone (GH) treatment induces insulin resistance of protein metabolism and consequently reduces the theoretical possibility for increased protein synthesis in the fed state. GH markedly reduces the amount of carcass fat; consequently the quality of products increases. Nevertheless, side effects of continuous GH administration by implants are frequent, as increased glucose or insulin levels [41]. GH transgenic animals developed problems as lethargy, lameness, gastric ulcers and anoestrus [42].

Life tide ® SW5 is the world`s first and only approved growth hormone releasing hormone (GHRH) DNA therapy for food animals. It is an injectable DNA plasmid encoding for porcine GHRH and administered once for a life time treatment in sows of breeding age not only to increase the growth rate but also to increase number of piglets born alive and weaned [43]. After intramuscular injection and electroporation the active constituent Life tide ® SW5 plasmid sequence enters to the skeletal muscle cell at the injection site and resides within the muscle cell [44]. Then the treated muscle cells actively produce GHRH at physiological concentrations. The GHRH induces the animal to produce and secret indogenous growth hormone under the control of normal physiological feedback mechanism [45]. The use of GHRH, the upstream stimulator of GH, was considered to be an alternate strategy that may increase not only growth performance or milk production for large species such as pigs or cattle, but also more importantly, the efficiency of production from both practical and metabolic perspectives [13]. GHRH therapy seems to be more physiological than GH therapy, as interaction between GH and its receptor suggests that the molecular heterogeneity of circulating GH may have important implications in growth and homeostasis. GHRH cDNAs were characterized in porcine, bovine and many other species. It is well established that extra cranially secreted GHRH as mature peptide or truncated molecules can be biologically active and even produce acromegaly in humans in a wide range of dosage [46].

CONCLUSION

Gene therapy is an emerging field of research and development that seeks new solutions to pressing health and environmental problems by combining physical sciences and engineering, with life sciences and medicine. Gene therapy represents the future of medicine and health but its growth is slow in the field application. The use of molecular and cellular tools to genetically modify and improve food supply is playing a leading role in the continuing concern to produce more food and of a better quality for the increasingly demanding world population. The viral vectors and plasmids are capable of genomic integration that can be used effectively in *ex vivo* protocols to achieve long-term expression and avoid exposure of the host to viral proteins.

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