Biopharming - A New Hope for Pharmaceutical Proteins Production
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Abstract

Animal biotechnology is one of the fastest growing sectors in twenty-first century. Transgenic animals produced by introducing ‘foreign’ DNA into the host genetic material have greater flexibility in direct genetic manipulation of livestock. Numerous potential applications of this methodology include increased growth rate, improved feed usage, improved carcass composition, increased disease resistance, enhanced reproductive performance and overall genetic improvement of livestock. Biopharming, one of the important applications of transgenic technology, is the method of production of recombinant proteins in transgenic livestock. The recombinant proteins can be collected from different body secretion i.e. milk, urine, seminal plasma, egg etc. So biopharming may prove to be a useful method in near future for production of different proteins that are highly essential for human beings.

Key words: Biopharming, Transgenic Animals, DNA, Recombinant Proteins

Introduction

Biotechnology is the utilization of biological systems for scientific discoveries and production of goods and services that improve the human environment. Biopharming involves large scale production of recombinant proteins by using transgenic animals. In a transgenic animal, foreign DNA has been incorporated so that their DNA is altered producing proteins that normally they would not produce. This concept was first developed by Briggs and King (1952) and later Gordon and Ruddle (1981) produced first transgenic mouse by DNA microinjection method. Since then there has been rapid development in the use of genetically engineered animals and constant efforts are made to improve the efficiency of generating transgenic livestock (Hyttinen et al., 1994; Kopchick et al., 1996).

How to Generate Transgenic Animals?

Several methods have been tried for transgenic animal production through the years (Irvine, 1991; Wheeler, 2003; Zaman, 2013). All these protocols include two fundamental aspects of transgenic technology: the generation of recombinant DNA suitable for transgenic expression and the techniques employed to introduce it into animals.
The steps for production of ‘transgenic animals’ include -

1. Identification of sequence for the desired character
2. Characterization of sequence
3. Isolation of genes
4. Purification of genes
5. Cloning of genes in DNA construct
6. Transfer of construct to one cell embryo
7. Analysis of the efficiency of transferred gene

Methods of Gene Transfer in Farm Animals

Different methods were tried and out of them DNA microinjection, embryonic stem cell mediated gene transfer; retroviral mediated gene transfer and sperm mediated gene transfer are perhaps the most useful and the most successful methods.

DNA microinjection: DNA microinjection is the most efficient method for generating transgenic animal lines, but the rate of integration of foreign gene is very low. So, the cost of growing embryos in vivo is very high and in vitro fertilization and maturation can be practiced to reduce the cost. But in vitro grown embryos shows lower survival rate, so in vivo maturation of embryos is the best method till date (Jura et al., 1994).

Embryonic stem cell mediated gene transfer: Embryonic stem cells can be isolated from blastocyst stage, genetically manipulated and grown in laboratory conditions and can develop into fully formed adults (Yoisungnern, 2014). When these altered stem cells are microinjected into early stage of embryo (blastocyst stage), they produce ‘chimeric’ embryos of two or more distinct cell types that can be a constant source of recombinant proteins. Several experiments are continuously going on to develop embryonic stem cell lines for different livestock species.

Retroviral mediated gene transfer: The gene transfer can be achieved by vector generally a virus or plasmid. Retroviruses are commonly used vectors to transfer genetic material into the cells and offspring produced by this method are chimeric (Archer et al., 1994).

Sperm-mediated gene transfer: Sperm-mediated gene transfer (SMGT) is based on the intrinsic ability of sperm cells to bind and internalize exogenous DNA molecules and to transfer them into the oocyte during fertilization. The major benefits of the SMGT technique are found to be its high efficiency, low cost and ease of use compared with other methods. For pharmaceutical applications (e.g. xenotransplantation) which require extensive genetic manipulation of donor (e.g. pig) sperm-mediated gene transfer can be used.

Production of Recombinant Proteins by Different Animal Systems
Though, application of genetic engineering allowed production of different proteins in recombinant bacteria, still, some proteins are either produced in very less amount or cannot be synthesized in active form and hence must be obtained from recombinant animal cells. But animal cells in culture generally do not produce large quantity of recombinant proteins due to lack of sufficient number of viable cells. Therefore, development of transgenic technology can be helpful to overcome these difficulties and lead to bulk production of recombinant proteins in animal bioreactors (Costa et al., 1990; Hoyer et al., 1994; Jänne et al., 1998; Echelard, 1996). The first transpharmer, a mouse, was engineered in 1987 to express the tissue plasminogen activator (tPA) (Gordon et al., 1987). Larger animals like sheep, goats, and cows can be targeted for large-scale transpharming. The first transgenic sheep created in Roslin Institute produced a human clotting factor in their milk. Different animal systems used for production of recombinant proteins are blood, urine, seminal plasma, egg white, milk etc. Although blood can be used for collection of recombinant proteins synthesized in specific tissues but the major limitations are difficulty in separation of recombinant from the endogenous proteins and many proteins are poorly stable in blood. Milk is currently the best available bioreactor (Dalrymple and Garner 1998) as it is easier to acquire the drug from milk, and proteins expressed in milk are less likely to affect the animal’s physiology than in the blood (Wilmut and Whitelaw, 1994). The proteins which can be collected are: insulin like growth factor, growth hormone, lysozyme, lactoferrin, fibrinogen, collagen etc. Mostly these proteins have been secreted in a fully functional form in milk (Rosen et al., 1996; Wall et al., 1991; Krimpenfort, 1993; Maga et al., 1994; Karatzas, 2003). An exotic protein, spider silk (Biosteel), known for its exceptional mechanical strength has been produced in milk (Williams, 2003). Urine can be used to collect recombinant proteins (e.g. gonadotropins) for pharmaceutical use (Meade and Ziomek, 1998). The human growth hormone gene can be expressed specifically in urothelium of mouse and collected through urine. Seminal plasma can be used to collect human growth hormone from transgenic mice. Similarly, transgenic bird can be developed which will secrete recombinant proteins through egg white.

Which Recombinant Proteins to Be Produced?

The gene coding for recombinant proteins should be selected on the basis of scientific, economic and social realities (Houdebine, 2002; Houdebine, 2000; Dave and Bruley 1999; Kling, 2009; Brüggemann et al., 2014). Tissue specific expression of recombinant proteins is more advantageous than generalized expression as it will be easier to regulate the gene expression in a tissue specific manner (Larrick and Thomas, 2001). For example, promoter elements derived from the casein and whey families have been used to direct expression and secretion of the recombinant proteins in the milk of cows, sheep and goats (Rudolph, 1999).
Another important issue in production of recombinant proteins is whether the foreign protein will have any effect on physiology of transgenic animal (Palmer et al., 2003; Faizi, 2013). Some pharmaceutical proteins exhibit adverse effect in transgenic animals, though their number is very less.

**Limitations of Biopharming**

Though, the transgenic procedure sounds very promising, but very expensive, and still has a low rate of success (Houdebine, 1994; Brink et al., 2000), especially for larger farm animals. The transgenic organisms producing recombinant proteins show some unexpected results like impaired mammary gland development, agalactia, gastric ulcer, arthritis, infertility and premature death (Ward et al., 1989; Plump et al., 1992; Colman, 1996; Sugiyama, 1997; Hathaway and Shur, 1996; Shamay et al., 1992; Lezauna and Porter, 2014). So before using transgenic techniques as a viable industry for production of recombinant proteins every aspect of its sustainability, profitability and animal ethics should be thoroughly examined. Some companies producing recombinant proteins using transgenic livestock are listed below in the table. Further improvements are needed to be done to increase efficiencies and economics of production of recombinant proteins considering scientific and regulatory difficulties simultaneously.

<table>
<thead>
<tr>
<th>Company</th>
<th>Recombinant protein</th>
<th>Livestock</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematech</td>
<td>Human monoclonal antibodies</td>
<td>Cattle</td>
</tr>
<tr>
<td>GTC biotherapeutics</td>
<td>Human serum albumin</td>
<td>Cattle</td>
</tr>
<tr>
<td>PPL</td>
<td>Alpha 1 antitrypsin</td>
<td>Sheep</td>
</tr>
<tr>
<td>GTC biotherapeutics</td>
<td>Antithrombin III</td>
<td>Goat</td>
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<tr>
<td>Nexia biotechnologies</td>
<td>Spider silk protein and human butyrylcholinesterase</td>
<td>Goat</td>
</tr>
<tr>
<td>Vivalis, Virage</td>
<td>Recombinant protein</td>
<td>Chicken</td>
</tr>
</tbody>
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**References**


