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Minireview II

TRANSGENIC RABBITS - MAMMARY GLAND AS A TARGET OF THE TRANSGENE

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ABSTRACT

The mammary gland is the most promising target tissue because it produces large amounts of recombinant human proteins, enzymes, hormones and growth factors in a temperature-regulated fluid that may be collected daily, in a non-invasive fashion. Different observations and conclusions have been made concerning the development, histology and ultrastructure of transgenic mammary epithelium. Therefore, there is a necessity to examine possible histological and ultrastructural changes in transgenic rabbit mammary gland tissue. This review summarizes recent results based on the analyses of transgenic rabbit mammary gland at different developmental stages over four generations of transgenic animals.

Key words: transgenic rabbit, mWAP-hFVIII gene, mammary gland, histology, ultrastructure

INTRODUCTION

Mammary gland as a "bioreactor"

The technology for using the mammary gland as a bioreactor has been developed to the point that pharmaceuticals derived from the milk of several transgenic farm animal species are currently in the advanced stages of clinical trails (Dove 2000; Fan 1999; Bosze 2003; Chrenek and Makarevich, 2008b). Recombinant proteins can be produced in prokaryotic or eukaryotic systems, so that they are derived from bacterial cells, yeast cells, transformed plant and animal cells, and even from live bioreactors (transgenic animals). Difficulty in obtaining transgenic individuals producing high levels of recombinant, biologically active proteins in required form with subsequent washing and clinical testing are the largest disadvantages of using farm animals for such purposes.

The use of transgenic animals allows the production of valuable human proteins, enzymes, hormones and growth factors in the milk. By targeting the expression of the transgene product to the secretory cells of animals we can collect and process body fluids with minimal effort. Transgenic rabbit system is a low-cost alternative primarily because rabbits are smaller and thus less expensive to maintain but also because the rabbit reproductive cycle is much shorter than that of the large domesticated animals (Bozse et al., 2003).

The mammary gland is the most promising target tissue because it produces large amounts of protein in a

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temperature-regulated fluid that may be collected daily, in a non-invasive fashion. Production of recombinant proteins in the mammary gland of transgenic animals is dependent on gene promoters used in transgene constructs. Most of the studies have been carried out with the ovine ß-lactoglobulin, bovine α-lactalbumin, caprine ß-casein or mouse whey acidic protein (WAP) promoter (Pollock et al., 1999). The mouse WAP promoter has been used in basic biological studies, as well as for the synthesis of pharmacologically active human proteins, to direct the expression of heterologous genes to alveolar epithelial cells (Van Cott et al., 2001; Chrenek et al., 2002; 2005; 2007; Palmer et al., 2003).

The lactation specificity of the regulatory regions used in control of mammary gene expression in transgenic animals is very important in case of expression of foreign biologically active proteins, because these proteins may exhibit their biological functions in the animal if secreted prior to the tight junction formation in the mammary epithelial cells (Chen et al., 2002). The effects of expressing growth factors, hormones, oncogenes, ECM components and receptors on mammary gland development and differentiation have been well documented (Jhappan.et al., 1993). These effects range in phenotype from impaired lubulo-alveolar development (Jappan.et al., 1993) to decreased milk protein synthesis during lactation without effects on glandular epithelium in activated Ha-ras mice (Andres et al., 1988), constitutive milk protein gene transcription in c-myc mice (Andres et al., 1988), reduction in milk protein synthesis and lactation deficiency in transgenic mice (Nemir et al., 2000), to premature dedifferentiation of secretory epithelial cells and a decrease in milk protein mRNA levels in beta1-integrin mice (Faraldo et al., 2002), to hyperplasia and delayed involution in human FGF4 transgenic mice (Morini et al., 2000).

Extensive studies of the effects of recombinant proteins on animals itself and on several generations must be performed before setting up a herd for production purposes. The majority of expressed recombinant proteins in transgenic animals does not cause negative effects on mammary gland structure and function. In cases where proteins with potent biological activities have been expressed, like erythropoietin, systemic deleterious effects have been observed, probably due to "leakage" of protein into the circulation (Massoud et al., 1996). Recently, some negative effects of WAP-human protein C transgene overexpression on the development of the mammary gland in homozygous transgenic mice were recently reported (Palmer et al., 2003).

The mammary gland is one of relatively few organs in the body that undergoes repeated cycles of structural development, functional differentiation, and regression (Hennighausen et al., 1997). Mammary gland morphogenesis is facilitated by a precise sequence of cell-cell and cell-matrix interactions, mediated in part through cell surface receptors and their ligands (Boudreau et al., 1995).

There have been few scientific studies addressing this problem with several of the following authors presenting similar results. Christa et al. (2000) conducted research on the expression of the human hepatocarcinoma-intestine-pancreas/pancreatic-associated protein (*HIP/PAP*) gene in the mammary gland of lactating transgenic mice. In order to ascertain whether the presence of HIP/PAP or its accumulation in the mammary cells had any effect on the mammary gland, the authors compared the ultrastructure of mammary epithelial cells from HIP/PAP lactating transgenic mice to that of normal lactating mice. No major changes to the morphological aspect were observed in the mammary epithelial cells from transgenic mice. However, numerous distended Golgi apparatus and secretory vesicles, both containing an elektron-dense and filamentous material, were observed, when compared to wild-type mouse mammary cells. This is probably due to the type of secretory product, as this was not observed with proteins like growth hormone and alpha1-antitrypsin (Devinoy et al., 1995).

Histology and ultrastructural morphology of the transgenic mammary gland

Different observations and conclusions have been made concerning the ultrastructure of transgenic mammary epithelium. Histological analyses of the mammary tissue of HPC/PACE bigenic mice did not reveal any differences in their gross morphology when compared with control mice, although the transgenic tissues differed subtly, having less distended alveoli with larger epithelial cells (Paleyanda et al., 1997), as also seen in hPC single transgenic mice (Paleyanda et al., 1994). Other authors have conducted similar experiments with different species of transgenic animals. Chen et al. (2002) investigated the presence of bio-active hFVIII in freshly dissected mammary gland tissue samples, using an immunohistochemical assay. This experiment showed that transgene products accumulated within mammary acini, as well as in the lumen of lactiferous tubules, and in that way it could have influenced cell volume. On the other hand, Palmer et al. (2003) recently reported that two lines of homozygous transgenic mice overexpressing recombinant human protein C under the control of the mouse WAP promoter, exhibited defects in lactation and impaired mammary gland development. Histological analyses during lactation showed barely distended alveoli filled with densely-staining milk. Females of both lines had normal growth, activity and fertility, but failed to lactate normally and were unable to raise litters, while hemizygous animals derived from these lines were able to lactate and raised normal-sized litters. This suggests

a gene dosage effect or an effect of the level of protein produced.

The whey acidic protein gene has been found to be expressed specifically in mammary tissue at late pregnancy and throughout lactation. Expression of mouse WAP protein in transgenic pigs impaired mammary gland development (Shamay et al., 1992). These results were confirmed by observations of Nukumi et al., (2004), where overexpression of WAP transgene impaired the lobulo-alveolar development of the mammary gland of transgenic mice and showed that WAP played a regulatory role in the cell cycle progression of mammary epithelial cells. Gene dosage and protein compartmentalization may also contribute to these effects. Homozygous transgenic females from multiple lines overexpressing the long isoform of cell surface receptor beta1,4-galactosyltransferase (GalTase) failed to lactate, whereas transgenic mice overexpressing the Golgi-localized short isoform of GalTase lactated normally (Hathaway and Shur, 1996). Glands from surface GalTase transgenics were characterized by abnormal and reduced ductal development with a concomitant reduction in alveolar expansion during pregnancy. Morphological changes were accompanied by a dramatic reduction in the expression of milk-specific proteins, suggesting that GalTase was important for normal mammary gland differentiation.

The mammary gland histology of transgenic rabbits producing recombinant human factor VIII in comparison with that of non-transgenic rabbit females with a several significant differences were reported (Dragin et al., 2006; Chrenek et al., 2009). However, these results indicate no any harmful effect of the mWAP-hFVIII transgene expression on the state of mammary gland of transgenic rabbit females. The number and size of vacuoles are normally highly variable and in positive correlation with milk production. Reasons for differences in vacuole size in this experiment are unclear but could indicate minor changes in secretion or transport. Different size of mitochondria may suggest an alteration in the energy balance between two experimental groups or a different mechanism. Further studies on mammary tissue over several stages of lactation and involution are required to determine whether this is a constant feature. These results are interesting in light of the recent reports that reversible transdifferentiation of secretory epithelial cells into adipocytes occurs in the mammary gland (Morroni et al., 2004). Large, cristae-rich mitochondria with dense matrix were seen in secretory epithelium, unlike the elongated mitochondria of developing adipocytes.

Apoptosis in transgenic mammary gland

Proliferation of mammary gland cells is an important determinant of milk yield. Opposite characteristics to proliferation is a programmed cell death – apoptosis. Through apoptosis each organ or tissue in organism eliminates excessive or damaged cells (Makarevich et al., 2008). In cows, goats and mice significant amounts of apoptotic cells were detected in the mammary gland during the lactation, and changes in mammary DNA content were accompanied by loss in milk production during prolonged lactation (Capuco et al., 2001; Hadsell et al., 2005). In the mammary gland of lactating rabbit females no any differences in the number of apoptotic cells between transgenic and non-transgenic animals were found (Chrenek et al., 2009). However, during the involution of mammary gland a rate of apoptotic cells in non-transgenic females was increased as compared to the transgenic ones (Chrenek et al., 2009). The fact, that apoptotic cells were less apparent in the mammary tissue of transgenic rabbits during the involution stage, supports our earlier finding that the mWAP-hFVIII gene construct does not bring any negative effect on transgenic rabbit milk performance (Chrenek et al., 2007a; 2007b). Since the process of the mammary gland involution involves dedifferentiation of epithelial cells to non-secreting cells, it may be accompanied with an increased rate of apoptosis in this tissue. Probably increased rate of apoptotic cells in non-transgenic tissue, as observed in our study, reflects existing difference in the dynamics of involution process compared to transgenic tissues. So far, no reports about apoptosis in transgenic mammary gland tissue are known, therefore clarification of this process in the mammary gland affected by targeted transgenesis must await further investigations.

CONCLUSION

Production of recombinant proteins in the mammary gland of transgenic animals is dependent on gene promoters used in gene constructs. Basing on the type and size of gene constructs and animal species different observations have been done in concern to the development stage, histological and ultrastructural status of transgenic mammary gland tissue. Prior to application of transgenic animals for recombinant protein production, there is a necessity to monitor also possible qualitative and quantitative changes in mammary gland in order to eliminate transgenic animals with undesirable alterations, which can reduce efficiency of transgenesis.

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