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Pathology Phenotyping of Transgenic Mice for Modelling Human and Animal Diseases

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Abstract. During the last 30 years, spontaneous mutations in mice mimicking a wide variety of human diseases have been collected for research purposes. The use of genetically engineered mice has been considered one of the most important advances of the 1990s, the so called "decade of the mouse", that will clearly improve in the twenty-first century taking into account the biomedical research revolution in technology.

INTRODUCTION

Genetic modification has been developed in plants and animals being the mouse, by far, the most widely used. Several technological advancements preceded the production of the first transgenic mouse reported in 1980 (Gordon J.W. *et al*).

- 1963 (Brinster, R.L.) culture of mouse embryos,
- 1966 (Lin T.P.) development of microinjection techniques,
- 1986 (Palmiter, R.D. & Brinster, R.L.) good knowledge of mouse reproductive physiology,
- 1992 (Cohen *et al*) development of recombinant DNA technology.

Next step was to develop a giant transgenic mouse incorporating in its genome the rat genomic sequence codifying for the synthesis of growth hormone (Palmiter *et al*, 1982). Actually, this possibility of efficient expression of a gene from different specie is the base for the development of transgenic livestock.

The objectives for the use of transgenic animals are:

- To improve the productivity.
- To create genetic resistance to diseases, as celiac disease (de Kauwe A.L. *et al*, 2009).
- To obtain human therapeutic proteins from animal origin or "transgenic farming", as the synthesis of albumin in dair
- y cow milk (Echelard Y., 2009).
- To "humanize" animals as donors of tissues and organs in xenotransplantation (Lanza *et al*., 1997; Cooper *et al*., 1997).

- To dispose of good models of human and/or animal diseases, as an example, transgenic mice are widely used as models of spongiform encephalopathies in animals and humans (Scott M. *et al*, 1993; Béringue V. *et al*, 2008).

The advantages for the use of the mouse as model are multiple:

- As mammals, mice and humans have biochemical resemblance.
- Its reproductive cycle is very short (19-21 days) and its litter large (12-15 newborns).
- Mice have a handy size which makes it cheaper to keep and process large populations.
- Most research centres or institutes have good facilities for mice husbandry.
- Its purchase and husbandry is cheaper than that of most laboratory animals.
- There are many transgenic strains and well known targeted mutant mice available for research.
- Genetic map of mice and humans is better known than that of other species.
- The use of genetically engineered mice could dramatically decrease the number of animals and the costs of procedures in research work, as is the case of the use of TgAC transgenic mice in carcinogenesis protocols (Humble, M.C. *et al*, 2005).

Initially, transgenic strains were utilized to characterize regulatory mechanisms of mammalian gene expression (Ramírez A. *et al*, 1994, 1995, 1998, Casanova LL. *et al*, 1995), they now provide models for human and/or animal diseases. In fact, the development of genetically engineered mice in biomedical research is considered the most important tool to discover the mutant phenotype or pathological consequences of a natural or induced genotype.

The clinical and pathological appearance of a new mouse depends on gene expression in specific cells, tissues, and organs. Inactivation of a gene or overexpression of the gene and its protein may cause changes in cell function, tissue and organ development, and cell and tissue differentiation. These changes can produce abnormalities in clinical appearance and gross and microscopic pathology (Ward, J.M., 2000). These abnormalities may produce a specific mouse phenotype recapitulating a human disease.

To determine the utility of a new genetically engineered mouse, either targeted mutant, transgenic, or their types or combinations, as a model of a human or animal disease, or for studying mechanisms of disease and/or function of a gene, it is important to determine the phenotypic characteristics, clinical and pathological, of the new mutant mouse line to ensure that it is used properly as a research tool (Ward, J.M., 2000).

The establishment of phenotypic features in a genetically engineered mouse is dependent upon appropriate longitudinal studies of the given mice in comparison with age-matched littermates with normal genotypes. These studies need multidisciplinary application of knowledge and techniques in Molecular Biology, Biochemistry, Anatomy, Physiology, Pathology and Surgery. Optimization of these procedures require relevant necropsy and tissue protocols for tissue sampling and sampling intervals, as well as active interaction between molecular biologists and veterinary pathologists with expertise in normal rodent gross and microscopic anatomy and pathology.

The first series of necropsies for a new mouse should be a complete screening of all tissues grossly and microscopically on selected mice. After lesions are found in some given

tissues, other specialized pathology methods should be used including histochemistry, immunohistochemistry, transmission and scanning electron microscopy, confocal microscopy, *in situ* hybridization and quantitative or stereological pathology (Ward, J.M., 2000).

Most models of disease are developed in induced mutants (knockout mice) and transgenic mice. Sometimes a new model of disease is discovered by accident, as occurs frequently in transgenic mice that integrate high copy numbers of the transgene usually resulting in high expression of a given protein. This is the case of HK8 transgenic mice (Casanova LL. et al, 1999) expressing the human K8 (*hk8*) gene, which leaded to a moderate keratin-content increase in their simple epithelia. These mice displayed progressive exocrine pancreas alterations, including dysplasia and loss of acinar architecture, redifferentiation of acinar to ductal cells, inflammation, fibrosis, and substitution of exocrine by adipose tissue, as well as increased cell proliferation and apoptosis. The phenotype found was very similar to human and animal chronic pancreatitis and indicate that simple epithelial keratins play a relevant role in the regulation of exocrine pancreas homeostasis and support the idea that disruption of mechanisms that normally regulate keratin expression *in vivo* could be related to inflammatory and neoplastic pancreatic disorders.

In some cases, a transgenic strain develops a pathological condition as a consequence of ectopic expression of a given protein. Transgenic mice expressing in hair follicles and epidermis a human keratin K8, normally expressed in simple epithelia, constitute a good model to examine the role of simple epithelium keratins in the establishment and progression of human skin cancer (Casanova LL. *et al*, 2004). These transgenic mice showed severe epidermal and hair follicle dysplasia with concomitant alteration in epidermal differentiation markers. The severity of the skin phenotype of these transgenic mice increases with age, leading to areas of preneoplastic transformation. Skin carcinogenesis assays showed a dramatic increase in the progression of papillomas toward malignancy in transgenic animals supporting the idea that K8 alters the epidermal cell differentiation, favours the neoplastic transformation of cells, and is ultimately responsible of the invasive behaviour of transformed epidermal cells leading of conversion of benign to malignant tumours.

Some transgenic mice constitute a model of inflammatory or immune-mediated diseases. IKKβ is a subunit of the IκB kinase (IKK) complex required for NF-κB activation in response to pro-inflammatory signals. NF-κB regulates the expression of many genes involved in inflammation, immunity and apoptosis, and also controls cell proliferation and differentiation in different tissues. K5-IKKβ transgenic mice with increased IKKβ activity in the skin constitute a model of lichenoid inflammation (Page *et al*, in preparation), a condition associated to important human and animal diseases, some of them potentially life-threatening, as dermatomyositis, erythema multiforme, lupus erythematosus, lichen planus, graft-versus-host reaction, and eruptions induced by drugs. The inflammatory phenotype observed as a consequence of IKKβ overexpression is independent of T and B lymphocytes, and also arises in the absence of a functional immune system. This study supports the idea that IKKβ might be a valid therapeutic target in the treatment of skin inflammatory diseases.

Also, the existence of these models allows the different laboratories to dispose of enough numbers of patients for the study of a variety of rare congenital non infectious diseases as is the case of targeted expression of Glucocorticoid receptor (GR) in the skin of K5-GR transgenic mice (Pérez P. *et al*, 2001, Cascallana *et al*, 2003, 2005). As a consequence

of transgene expression, these mice constitute a model of Hypohidrotic Ectodermal Dysplasia in humans and *aplasia cutis* in animals. K5-GR transgenic mice shows impaired development of the skin, hair follicles and ectodermally derived epithelia as teeth, eyelids, cornea and exocrine glands.

There is no doubt that in the third millennium, modelling human and/or animal diseases in genetically engineered mice is an essential tool to improve our knowledge about the pathogenesis and diagnose of important diseases and to develop new therapeutically approaches.

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