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Genetically Modified Animal Models; A Potential Cure for Cancer, Obesity, Heart Disease, Diabetes, Arthritis, Substance Abuse, Anxiety, Ageing and Parkinson's Disease

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ABSTRACT

Genetically engineered animal models have been used since 1973 as a tool for genetic research. Some of the areas of research have focused on the pathogenesis of different diseases, as well as their underlying mechanisms. The major type of animal models that have been used for this purpose is the "Knock-Out Mice". Knock-out mice are mice phenotypes resulting from inactivation of molecules (genes) responsible for the control of specific characteristics. Such modification usually leads to novel insights into the action of the deactivated gene action. This review will focus on nine different articles in which knock-out mice have been demonstrated to be useful in the study of treatment mechanisms for cancer, obesity, heart disease, diabetes, Arthritis, substance abuse, anxiety, ageing and Parkinson's disease.

INTRODUCTION

Cancer Treatment Research With Knockout Mice

p53 tumor suppressor gene therapy for cancer

The last two decades have led to a greater understanding of the genetic basis of human malignancy. "Numerous genetic alterations have been detected in cancer, but activation of onco-genes and inactivation of cell cycle regulators (e.g. tumor suppressor genes) are now known to play a critical role in the progression of the disease"[1].

Therapeutic strategies based on specific molecular alterations in cancer include reintroduction of wild-type tumor suppression function to cells lacking the gene. p53, a tumor suppressor gene whose activity stops the formation of tumors [2], can be used as therapy. p53 gene therapy powders an attractive strategy to test the potential clinical feasibility of this approach [1].

According to a number of studies, p53 gene mutations are the most frequent abnormality identified in human tumors and restoring its function can induce apoptosis in cancer cells. This article described various gene therapy strategies under investigation, reviewed preclinical data that provided a rationale for the gene replacement approach and discussed the clinical trial data available.

During this study[1], data was collected from preclinical documents for patients with ovarian and breast cancer. More data was obtained from already existing research data from genetic correction strategies in knock-out mice.

Results showed that gene therapy is a revolutionary step towards cancer treatment. Genetic correction strategies are being developed and tested in knock-out mice for human malignancies, and in early patient trials. The cancer susceptibility genes p53 were tested in ovarian and breast cancer patients and showed some potential for this antitumor strategy. p53 gene therapy may be effective even against tumors that lack p53 mutations because p53 may function as a growth inhibitor in a variety of gene transfer settings.

Obesity Treatment Research with Knockout mice

Cholecystokinin knock-out mice are resistant to high-fat diet induced obesity

Cholecystokinin (CCK or CCK-PZ) is a peptide hormone of the gastrointestinal system responsible for stimulating the digestion of fat and protein[3]. Cholecystokinin, previously known as pancreozymin, is synthesized by I cells, in the mucosal epithelium of the small intestine and secreted in the duodenum [3].

This research[4] sought to find out whether knockout mice with a disruption of CCK and fed on a diet of 20% butter fat would have altered fat metabolism.

A quantitative magnetic resonance imaging system was used to determine body composition and monitor food intake of CCK knock out mice using an automated measurement system. Intestinal fat absorption and energy expenditure were determined using a noninvasive assessment of intestinal fat absorption and an open circuit colorimeter, respectively.

CCK knock-out mice had reduced body weight gain and body fat mass and enlarged adipocytes, after consuming a high fat diet

for 10 weeks, despite receiving the same level of food intake as wild-types mice (control). CCK knock-out mice also had defects in fat absorption especially of long chain saturated fatty acids, but pancreatic triglyceride lipase did not appear to have a role in the fat malabsorption. Energy expenditure was higher in CCK knockout mice than in wild-type mice, and CCK – knockout mice had greater oxidation of carbohydrates while on the high-fat diet. Plasma leptin levels in CCK knock-out mice fed the high fat diet were markedly lower than in wild-type, although levels of insulin, gastric-inhibitory polypeptide, and glucagon-like peptide 1 were normal.

These results showed that CCK is involved in regulating the metabolic rate and is important for lipid absorption and control of body weight in mice placed on a high – fat diet.

Heart Disease Treatment Research with Knockout mice

Targeted deletion of the 9p21 non-coding coronary artery disease

9p21 is a chromosome which is physiologically responsible for risk to cardiovascular disease and is therefore a potential therapeutic target in cardiovascular medicine. This genetic variant accounts for more than 50% of susceptibility of coronary artery disease [5].

A robust association between a large intergenic locus on chromosome 9p21 and the risk of coronary artery disease was reported[5]. The finding was that the prevalence of the risk allele was higher in patients with severe premature atherosclerosis than in patients with incident coronary artery disease (which generally occurs at a later stage).

It was suggested that this allele promotes coronary artery disease by accelerating atherosclerosis. Other studies also found the 9p21 locus to be associated with the risk of stroke, peripheral-artery disease, abdominal aortic aneurysm, and, intriguingly, intracranial aneurysm. The latter finding points to possible effects on vascular remodeling pathways"[5].

This article sought to find the mechanistic basis for the association between 9p21 risk alleles and coronary disease. It was hand demonstrated that the 9p21 is associated with a duster of cell-cycle regulating genesis whose functions involved: Cell proliferation, aging and apoptosis and are well demonstrated in tumor-suppressor genes [5].

This study sought to find whether generic features of the 9p21-related risk interval in coronary disease modulate expression of these cell-cycle regulator genes.

To answer this question, a 70-kb non-coding region on mouse chromosome 4, which is orthologous to the human 9p21 risk interval was deleted [5].

A markedly decreased expression of the cell-cycle regulator genes was observed in the mutant mice. They also observed a doubling of the proliferative capacity of mutant aortic smooth muscle cells in culture, a cellular phenotype relevant to atherosclerosis.

Consistent with the tumor-suppressor effect of the cell-cycle regular genes, tumors developed in close to half the knock out mice.

Arthritis Treatment Research with Knockout mice

Suppression of inflammation by low-dose methotrexate is mediated by adenosine A_{2A} receptor but not A_3 receptor activation in thioglycollate-induced peritonitis

Adenosine, acting at one or more of its receptors, mediates the anti-inflammatory effects of methotrexate in animal models of both acute and chronic inflammation [6]. Both adenosine A_{2A} and A_3 receptors contribute to the anti-inflammatory effects of methotrexate treatment in the air pouch model of inflammation, and the regulation of inflammation by these two receptors differs at the cellular level [6].

In this study, the effects of low-dose weekly methotrexate treatment (0.75 mg/kg/week) in a model of acute peritoneal inflammation in adenosine A_{2A} receptor knockout mice and A_3 receptor knockout mice and their wild-type littermates were examined to find out the if different factors were responsible for regulation of inflammation at different sites.

Following intraperitoneal injection of thioglycollate there was no significant difference in the number or type of leukocytes, tumor necrosis factor alpha (TNF-α) and IL-10 levels that accumulated in the thioglycollate-induced peritoneal exudates in adenosine A_{2A} knockout mice or wild-type control mice. In contrast, there were more leukocytes, TNF-α and IL-10 in the exudates of the adenosine A3 receptor-deficient mice. Low-dose, weekly methotrexate treatment increased the adenosine concentration in the peritoneal exudates of all mice studied, and reduced the leukocyte accumulation in the wild-type mice and A_3 receptor knockout mice but not in the A_{2A} receptor knockout mice. Methotrexate reduced exudate levels of TNF-α in the wild-type mice and A_3 receptor knockout mice but not the A_{2A} receptor knockout mice. More strikingly, IL-10, a critical regulator of peritoneal inflammation, was increased in the methotrexate-treated wild-type mice and A3 knockout mice but decreased in the A_{2A} knockout mice. Dexamethasone, an agent that suppresses inflammation by a different mechanism, was similarly effective in wild-type mice, A_{2A} mice and A_3 knockout mice.

These findings provided evidence that adenosine is a potent regulator of inflammation that mediates the anti-inflammatory effects of methotrexate. Moreover, these data provide strong evidence that the anti-inflammatory effects of methotrexate and adenosine are mediated by different receptors in different inflammatory loci, an observation that may explain why inflammatory diseases of some organs but not of other organs respond to methotrexate therapy.

Anxiety Treatment Research with Knockout Mice

The 5-HT (1A) receptor knockout mouse and anxiety

The 5-HT (1A) receptor has been implicated in the modulation of anxiety processes, mainly via pharmacological experiments. The recent production, in three independent research groups, of 5-HT (1A) receptor knockout (R KO) mice in three different genetic backgrounds (C57BL/6J, 129/Sv, Swiss-Webster) led to the intriguing finding that all mice, independent from the genetic background strain from which the null mutants were made, showed an "anxious" phenotype compared to corresponding wild-type mice [7].

This research reviewed the behavioral findings in these three KO lines and focused on new findings in the 129/Sv-KO mice.

These mice were more anxious or stress-prone only under specific conditions (high stress). The 5-HT (1A) R KO made in the Swiss-Webster background displayed disturbances in the

GABA (A)-benzodiazepine (BZ) receptor system in the brain, including down regulation of GABA (A) alpha1 and alpha2 subunits in the amygdala. In contrast, the GABA (A)-BZ receptor system seemed to function normally in the 5-HT(1A) R KO in the 129/Sv background suggesting that changes in the GABA(A)-BZ receptor system may not be a prerequisite for anxiety but rather could have a modifying effect on this phenotype.

It was concluded that the constitutive absence of the 5-HT (1A) receptor gene and receptor leads to a more "anxious" mouse, dependent on the stress level but independent from the strain. Depending on the genetic background, it was concluded that this null mutation may be associated with changes in GABA (A)-ergic neurotransmission.

Substance Abuse Treatment Research with Knockout mice

Dramatically Decreased Cocaine Self-Administration in Dopamine but not Serotonin Transporter Knock-Out Mice

There had been much interest in the relative importance of dopamine and serotonin transporters in the abuse-related-effects of cocaine.

In this study[8], the tested hypothesis was that mice lacking the dopamine transporter (DAT–/–), the serotonin transporter (SERT–/–), or both (DAT–/–SERT–/–) exhibited decreased reinforcing effects of cocaine. It was also assessed whether observed effects on self-administration are specific to cocaine or if operant behavior maintained by food or a direct dopamine agonist are similarly affected.

A broad range of experimental conditions was used. This included acquisition without previous training, behavior established with food training and subsequent testing with food, cocaine or a direct dopamine agonist as reinforcers, fixed ratio and progressive ratio schedules of reinforcement, and a reversal procedure.

Wild-type mice readily acquired cocaine self-administration and showed dose–response curves characteristic of the schedule of reinforcement that was used. While some DAT–/– mice appeared to acquire cocaine self-administration transiently, almost all DAT–/– mice failed to self-administer cocaine reliably. Food-maintained behaviors were not decreased by the DAT mutation, and IV self-administration of a direct dopamine agonist was robust in the DAT–/– mice. In contrast to those mice, cocaine's reinforcing effects were not diminished in SERT–/– mice under any of the conditions tested, except for impaired initial acquisition of both food- and cocaine-maintained behavior.

These findings supported the notion that the DAT, but not the SERT, is critical in mediating the reinforcing effects of cocaine.

Ageing Treatment Research with Knockout mice

Anti-aging Research Using Mn-SOD Conditional Knockout Mice

Manganese superoxide dismutase (Mn-SOD) is a mitochondrial enzyme that converts toxic O (2) (-) to H (2) O (2) [9]. Previous studies have reported that a systemic deficiency in Mn-SOD causes neonatal lethality in mice. Therefore, no mouse model is available for the analysis of the pathological role of O (2) (-) injuries in adult tissues [9].

In order to explore an adult-type mouse model, this study by Yakugaku, 2010 sought to generate tissue-specific Mn-SOD conditional knockout mice using a Cre-loxp system.

First, they generated liver-specific Mn-SOD-deficient mice by crossbreeding with albumin-Cre transgenic mice. Mn-SOD proteins were significantly down regulated in the liver of liver-specific Mn-SOD knockout mice.

The mutant mice showed no obvious morphological abnormalities or biochemical alterations in the liver, suggesting a redundant or less important physiological role for Mn-SOD in the liver than previously thought.

Next, they generated heart/muscle-specific Mn-SOD-deficient mice by crossbreeding with muscle creatine kinase-Cre transgenic mice.

The mutant mice developed progressive dilated cardiomyopathy with specific molecular defects in mitochondrial respiration. Furthermore, skeletal muscle-specific Mn-SOD-deficient mice that had been generated by crossbreeding with human skeletal actin-Cre transgenic mice developed a severe physical disturbance associated with impaired cellular ATP metabolism.

These results implied that the superoxide generated in mitochondria plays a pivotal role in the development and progression of pathologies in the heart and skeletal muscle, but not in the liver.

In conclusion, various tissue-specific Mn-SOD conditional knockout mice that provided useful tools for the analysis of various oxidative stress-associated diseases were successfully generated.

Parkinson's Disease Treatment Research with Knockout mice

A Nurr1-Knockout Mouse Model for Parkinson's disease and Stem Cell Differentiation

The researchers have generated Nurr1-knockout mice via genomic locus inactivation using homologous recombination. Transcription factor Nurr1 is an obligatory factor for neurotransmitter dopamine biosynthesis in ventral midbrain [10]. From a neurological and clinical perspective, it suggests an entirely new mechanism for dopamine depletion in a region where dopamine is known to be involved in Parkinson's disease[10]. Activation of Nurr1 may be therapeutically useful for Parkinson's disease patients; therefore, the mice would be useful in Parkinson's disease research[10]. Additionally, Nurr1 has been shown to be critical for development of midbrain dopaminergic neurons, and thus may contribute to stem cell-based therapies for neurological disorders. Nurr1 is also important for osteoblast differentiation, suggesting a general role in stem cell differentiation and growth [10].

Diabetes Treatment Research with Knockout mice

GPR39, A Novel Target for Type-2 Diabetes and Obesity

The GPR39 is a 7TM, G-protein coupled receptor, which is highly expressed in peripheral tissues with metabolic functions such as adipose tissue, liver, GI tract and the endocrine pancreas. GPR39 knockout mice has been shown to display normal insulin sensitivity but impaired glucose tolerance both during oral and intravenous glucose tolerance tests and decreased plasma insulin response to glucose challenge[11]. Furthermore, GPR39 knock out mice fed with high fat diet develops obesity faster than wild type littermates. It is suggested that GPR39 is involved in the

control of endocrine pancreatic function and fat accumulation and that this receptor could be a novel potential target for the treatment of obesity and diabetes.

CONCLUSION

The conclusions derived from these researches are limited by the fact that in the tissue- and cell type-specific knockouts, gene inactivation is effective throughout development. Therefore, 1) there are probably compensatory mechanisms for the loss of individual proteins, and 2) it may be difficult to distinguish phenotypes arising from developmental defects from those resulting from impaired signaling.

Nevertheless, mouse models with genetic defects can yield important information about the action of a that gene and are helpful in extending our understanding of the mechanisms underlying treatment of cancer, obesity, heart disease, diabetes, and Arthritis, substance abuse, anxiety, ageing and Parkinson's disease.

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