The “New” Genetics and Mammalian Cloning in Environmental Health Research

Recent advances in genetic technology have spurred a mini-revolution in the study of toxicology. Toxicologic studies are a national imperative, and the importance of the application of transgenic mice and knock-out technologies to these studies is widely recognized. For example, the use of Tg.AC transgenic mice, carrying an inducible v-H-ras gene, and p53-/- mice speeds the outcomes of the traditional 2-year bioassay of chemicals nominated for study (1-8). Mechanistic studies have been greatly enhanced by Big Blue transgenic animals that allow “shuttle” mutagenesis studies (9-11).

These genetic approaches have enhanced our knowledge of mechanisms that are important to molecular toxicology as well. By knocking out gamma-glutamyl transpeptidase, the paradoxical reduction of intracellular glutathione was found to be associated with the accumulation of DNA damage (12). Mechanistic roles for repair enzyme genes in toxicologic damage have been revealed with this technology. For example, mouse models of xeroderma pigmentosa produced by creating null mutations of xpc gene proved the critical function nucleotide excision repair by the xpc system in ultraviolet radiation-induced damage leading to skin cancer (13). By combining mutations, the overlapping roles of p53 (Tp53) and xpc, as well as base excision repair and mismatch repair, were revealed (14).

Similarly, this approach established the role of β-pol in long patch repair and established that the failure of this repair system can lead to chromosomal breakage and apoptosis (15,16). β-pol null cells were used to show that removal of 5’-deoxyribose phosphate moieties from DNA is a key step in base excision repair (17). The promise now is that knock-out technology, particularly combined with widespread application of gene array studies, will enhance the Environmental Genome Project goal of establishing mechanisms of gene-environment interaction (18).

The application of these technologies through model systems (fruitfly and Caenorhabditis elegans) that establish “the usual suspect” genes by sequence similarities was recently boosted with the completion of the Drosophila and C. elegans genome projects (19). These projects revealed a surprising level of sequence conservation to the human. In the case of Drosophila, sequence homology to humans is estimated to be approximately 50%, and > 60% of a subset of human disease genes (68% of human cancer genes) had orthologs in the Drosophila annotated genome. We know this conservation extends to important aspects of complete pathways as well, such as the Sonic hedgehog–Patched–GLI pathway (20).

The ability to take information from the model system to functional gene study with gain of function (e.g., transgenic) and loss of function (e.g., knock-out) mutations in analogous experimental systems such as the mouse is extremely powerful because of the genetic information available in mouse strains. It is important to remember that complete exploitation of this approach requires careful phenotypic analysis, which is often not available or difficult to obtain in the mouse.

Much of these data are already available or easily obtainable in the rat, however. Using the rat, physiologic and pathophysiologic data for common diseases and metabolic pathways have been gathered for nearly a century from models of diseases that are important to the national public health. Often the rat model most closely resembles the human from among acceptable experimental systems. Important rat models of human diseases include those for cardiovascular diseases, neurodegenerative diseases, behavioral disorders, metabolic disorders, and carcinogenesis, all of which have been targeted by the Toxicogenomics program (21-24).

The genomic resources for using rat models of human disease conditions are robust and growing rapidly (23,24). Particularly important in this regard is the recent announcement that the rat genome will be sequenced. Currently, over 97% of the rat genome is covered at high density by anonymous markers, and the rat expressed sequence tag project has about 60,000 National Center for Biotechnology Information (Bethesda, MD) UniGene clusters. Polymorphisms in genes relevant to human toxicologic exposure have been studied in the rat for many decades. Recently, there have been several national initiatives to establish centers to maintain and distribute important rat strains of known genetic and microbiologic quality, and these should be available to environmental health scientists in the near future. However, the full impact of these resources on mechanistic genetic studies is currently limited by the inability to produce knock-out rats. Indeed, at the time of this writing, knock-outs have only been successfully produced in the mouse.

This limitation may be overcome by establishing methods for nuclear transfer or cloning for the rat (25). Nuclear transfer is the process of removing the nuclear chromosomal material from unfertilized oocytes and replacing it with a nucleus from another cell, often an adult cultured cell (26). The nucleus reprograms, presumably by resetting methylated gene imprints, and the nuclear transfer oocyte can develop to term (27). The offspring from this process carries the genetic traits (including targeted mutations if present) of the cultured cells. Knock-out mice are currently produced by growing embryoid-derived pluripotent stem cells in culture, creating targeted mutations in them and then transplanting them into preimplantation mouse embryos to produce a chimera that comprises normal recipient lineages as well as lineages derived from the mutant cells. If the chimera has functional germ cells that are derived from the targeted cells, then the mutation can be bred from the chimeras to produce heterogeneous or homozygous mutant mice. As mentioned previously, this does not work in other animals because stem cells capable of producing germ line chimeras have not, so far, been reproducibly isolated except from certain strains of mice. On the other hand, nuclear transfer has been successful in producing clones of animals from several mammalian species (28-32), and results with the rat to date are encouraging. At this point it is possible to obtain advanced preimplantation stages from nuclear transfer rat oocytes, which is a prerequisite to developing animals. We have transfected embryonic fibroblasts with green fluorescent protein marker transgenes, and after selection, we used the cells as nuclear donors for nuclear transfer experiments. The genetically
modified rat cells are as efficient nuclear donors as unmodified cells are, so this method of making knock-out rats should work.

There are problems with this approach, however. The efficiency of producing cloned mammals appears to be approximately 2% of manipulated embryos, a number that seems not to be affected by the species used or the type of cell used as a nuclear donor. Currently, large-scale efforts to improve this efficiency are under way for several species of mammals. There is a high frequency of gestational loss of nuclear transfer fetuses with a wide variety of abnormalities, including high frequencies of placental abnormalities, regardless of the species used. The biochemical reprogramming required by the nucleus after transfer does not occur in a completely successful manner for the vast majority of embryos. Just what effect this would have on the phenotypic analysis of traits in animals produced this way is unclear. Nevertheless, measuring lines of animals with specific genetic traits in this way seems entirely feasible.

There are some very interesting questions that cloning raises independent of their potential utility in mechanistic genetic toxicology: What is nuclear reprogramming? What is the age of the animals relative to the age of the cells used as nuclear donors? What is the nature of the abnormalities in some of the clones? Why is the efficiency of cloning low and seemingly invariant? How do imprinted genes behave in cloned animals? What is the mechanism of methylation maintenance in cloned animals? Regardless of these questions and challenges, nuclear transfer offers the promise of providing a route to loss of function mutations in the rat. Nuclear transfer may provide the opportunity to exploit the marriage of phenotypic data, genomic data, expressed-gene data, and sequence conservation in pursuit of the goal of complete functional analysis of genes that modulate environmental exposure in human health. It is an exciting time for developing embryologic approaches to toxicology.

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