

Chicken Model

Poultry domestication from Red Jungle Fowl over the past 8,000 years has created not only a major global food source, but also a unique and very important animal model for biomedical research. Historically, for example, chickens have served as models for the study of embryology by Aristotle, transmission of infections by Pasteur, and trait selection in breed development by Darwin. Chickens were instrumental in early studies of genetics, vertebrate development, immunology, and medicine, and are still important models in these fields today. [1]

Easy access to and manipulation of chicken embryos from incubated eggs has traditionally been one of the greatest advantages of this animal model. However, now that the chicken genome has been sequenced, it is becoming a more and more powerful experimental system. The chicken genome has several advantages over others including its relatively small size and low frequency of repetitive DNA. Further, the chicken genome is being continually improved as more detail is added through bioinformatics and functional genomics studies. As a result of these features, the chicken model system is accelerating research in wide variety of fields including biomedicine. [1] While chicken is used in the study of a broad variety of human diseases, only a few will be highlighted here. Thus, this summary should be taken as neither comprehensive nor definitive, rather as an overview of some of biomedical fields in which chicken serves as an important experimental model.

Ovarian Cancer

Ovarian cancer (OC) is the most deadly of the gynecological cancers with nearly 70% of afflicted women dying from the disease. The high mortality rate is driven by the absence of early detection strategies and the frequent recurrence of OC that is resistant to available chemotherapeutic agents. Like most forms of cancer, early detection and treatment of OC significantly improves prognosis. Unfortunately however, most OC cases are only first discovered after the cancer has already progressed to later stages and spread throughout the abdomen. Lack of specific symptoms, biomarkers, and knowledge of early pathophysiological changes in the development of OC as well as the anatomical location of the ovaries deep in the abdomen hinder early detection efforts. Once detected, treatment consists of surgical removal of as much cancerous tissue as possible followed by chemotherapy. Sadly, current chemotherapeutic strategies are still inadequate as many patients fail to effectively respond and succumb to the disease. [2-4]

Further research is needed to solve both the detection and the treatment problems and pre-clinical research models will be critical to this effort as there are too few patients presenting with OC at early stages to provide adequate study numbers. Developing good, experimental animal models of OC is challenging because of our limited understanding of early disease as well as the relative paucity of animal models that spontaneously develop a human-like OC condition. Several rodent models have been created and are being used to study some aspects of OC, but because OC in rodents must be induced or genetically engineered and the tumors they develop are histopathologically dissimilar to those of humans, the translational relevance of this model is reduced. Large animal models such as bovine do spontaneously develop OC and have the advantage of sharing similarities with human reproductive physiology, but the feasibility of their use in this capacity is limited because bovine OC occurs at only a very low frequency. [2-4]

Perhaps the most exciting new experimental model of human OC is chicken as laying hens have several unique advantages that together offer a more complete approximation of human OC that is experimentally feasible. First, chickens spontaneously develop OC disease with high frequency – greater than 1 in 3 for hens relative to 1 in 70 lifetime risk for women. Second, chicken and human OC is very similar with tumors most often originating from ovarian surface epithelium (OSE), expressing markers such as EGF receptor (*a.k.a.* c-Met oncogene) and TNF- α , and displaying all four stages of tumor progression (FIGO classification stages I through IV). Further, in both species, OC tumors are associated with circulating anti-tumor antibodies and exposure to progesterone appears to be protective. Third, chickens share several important aspects of reproductive physiology with humans such as the development of 5 to 6 large hierarchical preovulatory follicles in a rapid ovulatory cycle controlled by pituitary gonadotropins and ovarian steroid hormones. Fourth, hens are readily available, easy to maintain, begin laying eggs at 20 to 22 weeks and reach peak production at 30 to 32 weeks. And finally, a naturally occurring point mutation found in layers reduces OC frequency by 9 fold. The strain bearing this sex-linked mutation, called the “restricted ovulator” (RO) chicken, expresses a functionally disrupted oocyte very-low-density lipoprotein (VLDL) receptor that results in reduced oocyte uptake of circulating yolk precursors and failure to properly develop and release normal oocytes. [2-4]

While human OC is almost certainly multifactorial with hormonal changes related to menopause or premature ovarian failure implicated in OC and as many as 8 documented environmental carcinogens capable of contributing to OC, one of the leading investigative paths into human OC tumorigenesis is the “incessant ovulation” hypothesis. This hypothesis is based the fact that roughly 90% of all human OC tumors originate from OSE, which is thought to actively participate in the ovulation process, and the idea that repeated rupture and repair of this tissue exposes OSE cells to increased risk of mitotic mutagenesis and therefore malignant transformation. The hypothesis is supported by epidemiological data showing protective effects of oral contraceptive use, pregnancies, extended periods of lactation, and early menopause, all of which drive reductions in numbers of lifetime ovulation events. It is further evidenced by the low incidence of OC in most non-human mammals, which generally spend most of their lives pregnant, lactating, or otherwise anestrous, similarly reducing numbers of ovulations. [2-4]

Using the chicken model, the “incessant ovulation” hypothesis has not only been reinforced, but has also been extended. Chickens under intensive laying conditions experience a high frequency of spontaneous, human-like OC disease, the incidence of which is reduced 9-fold when ovulation is restricted. Further, pre-cancerous, dysplastic, OSE lesions have been identified and described in chicken that appear similar to those observed in contralateral ovaries of OC patients suggesting that these represent very early OC. The chicken model offers extraordinary opportunities to further our understanding of the cellular and molecular mechanisms of OC initiation and progression and work toward diagnostic and therapeutic as well as screening and prevention solutions. [2-4]

Systemic Sclerosis

Systemic sclerosis (SSc), also known as scleroderma, is a rheumatic disease (closely related to rheumatoid arthritis and systemic lupus erythematosus) of the connective tissue that is chronic, progressive, and as yet, incurable. Although SSc is generally characterized by excessive skin fibrosis beginning in the extremities, chronic inflammation, and capillary dropout, it actually encompasses a very broad spectrum of presentations. SSc may be localized or systemic, it may involve one or multiple organs (most often skin, lungs, gastrointestinal tract, heart, and/or kidney), and it is often associated with Raynaud’s phenomenon, an over-activation of vasoconstrictor mechanisms in response to cold or stress that results in poor circulation to fingers and toes and eventual ulceration and necrosis. The

variation in clinical presentations, unknown etiology, largely undescribed early pathophysiological changes, and slow onset of SSc, together result in a high rate of initial misdiagnosis, late diagnosis, and generally poor prognosis. While symptoms may be managed via immunosuppressive or anti-inflammatory drugs, no pharmacological intervention is currently available to treat SSc directly. [5,6]

In the hope of uncovering novel therapeutic strategies for SSc patients, many researchers are focused on a few physiological mechanisms that appear to be potential interventional targets for SSc therapies. One of these is angiogenesis – SSc sufferers display reduced capillary densities and fail to replace damaged capillaries, suggesting that miss-regulation of angiogenesis is involved in SSc pathophysiology. These observations are strengthened by recent findings that a broad array of pro- and anti-angiogenic factors are differentially expressed in SSc patients relative to healthy subjects. Another of these mechanisms appears to be chronic inflammation – this aspect has provided SSc patients some relief from disease symptoms as anti-inflammatory and immunosuppressive drugs do have some ameliorative effects though do not halt or reverse the course of disease progression. Yet further example is autoimmunity – SSc patients carry a variety of auto-antibodies to nuclear antigens, anti-cardiolipin antibodies, anti-endothelial cell antibodies, and rheumatoid factors. [5,6]

The complexities that contribute to the difficulties in diagnosing SSc as well as the number of pathophysiological mechanisms implicated in disease progression make it challenging to establish animal models of the disease. As a result, most of the popular rodent models of human SSc can only approximate one or a few aspects of the disease rather than the whole. For example, the bleomycin-induced murine model is important for studying the chronic inflammatory component of the disease, but does not display either the vasculopathy or the autoimmunity aspects of SSc. Researchers using this model are investigating, for example, the contributions of the TGF- β /Smad pathway, effects of the chemokine MCP-1 and its receptor CCR2, and the balance between Th1 and Th2 responses. Unfortunately though, the model does not offer a very complete approximation of human SSc. [5,6]

By contrast, the University of California Davis chicken lines (UCD-200/206) offer the chance for researchers to examine vasculopathy, inflammation, and autoimmunity in the same animals. At only one to two weeks of age, chickens from these strains display a spontaneous, heritable disease that mimics human SSc and Raynaud's phenomenon in nearly every aspect. These animals develop microvascular occlusions, display massive recruitment of T lymphocytes to perivascular skin and visceral tissues, experience excessive accumulation of collagen types I, III, and VI resulting in significant skin thickening, suffer fibrosis of both skin and internal organs, and express auto-antibody profiles matching clinical observations. Because of the enormous similarities with human SSc, the UCD-200/206 chicken strains have already proven to be a good model for the disease and will be essential for discovering disease etiology, pathophysiology, and future life-saving therapies. [5,6]

Epilepsy

Epilepsy is a brain disorder that results in unpredictable and repeated seizures of a variety of forms. While the cause of the disease may sometimes be idiopathic, epilepsy often arises due to the effect of other medical conditions or brain injury. Unfortunately, currently available treatment strategies for epilepsy, including surgical intervention, medical device implantation, and pharmacotherapy, function almost exclusively to help manage symptoms rather than prevent or eliminate of the disease itself. Thus, patients are faced with life-long stresses associated with the unpredictability of potentially embarrassing seizures, impact of the disease on education, employment, and social connectedness, concerns regarding the potential dangers of using anti-seizure medications, increased potential for injury incurred

during seizures, and elevated risk of sudden death. Further, chronic stress renders epilepsy sufferers more prone to seizures, making living with the disease very frustrating. Basic, pre-clinical, and clinical research needs to be increasingly focused on prevention for high-risk individuals and definitive treatment for those already afflicted. The chicken model appears to have some relevance for the study of at least two aspects of epilepsy: genetic reflex epilepsy and sudden unexpected death in epilepsy. Use of this experimental animal model may provide important insights into epilepsy pathophysiology and contribute to the search for preventative and curative therapies. [7-9]

Genetic reflex epilepsy (GRE) is a form of epilepsy that results in seizures caused by sensory stimuli (most commonly visual or auditory) in genetically susceptible individuals. Photogenic and autogenic GRE, though not yet traced to a particular gene, behaves in an autosomal recessive fashion such that only homozygous individuals are affected. While GRE has been described in rats and baboons, Fayoumi strain chickens appear to perhaps be the best approximate of the human disease that is experimentally feasible. These chickens display human-like, photogenically-induced epileptic seizures in homozygotes (Fepi) and not heterozygotes (Fhtz). Intermittent light stimulation of these animals consistently produces seizures of predictable quality within the same individual and across affected individuals. Easy access to embryos from incubated eggs allows for study of development and anatomy of Fepi animals and their short maturation time facilitates more rapid genetics studies. Using this model, researchers have localized the possible origin of GRE to brain stem structures in the Fepi chicken and continue to work to uncover the genetic component behind this form of the disease. [8]

Epilepsy patients are at increased risk of death primarily due to sudden unexpected death in epilepsy (SUDEP), which accounts for 7.5% to 17% of all deaths of epilepsy patients. Risk factors for SUDEP include young age, early onset of epilepsy, duration of disease, antiepileptic drug therapy, and refractoriness of seizures to therapy. While the precise mechanisms of SUDEP are still unknown, coincident cardiac arrhythmias have been implicated. To examine this problem further, researchers have turned to animal models including broiler chickens, which are susceptible to sudden death syndrome (SDS). SDS occurs when apparently normal, healthy chickens display a sudden bout of violent wing flapping lasting less than a minute and then abruptly die. A range of nutritional and environmental factors have been suggested as triggers for chicken SDS, but like human SUDEP, the etiology is unknown and cardiac arrhythmias are implicated. Further studies in chickens have suggested that chronic stress leads to fatal ventricular arrhythmias and death in SDS. Broilers in commercial production situations are subjected to constant environmental, metabolic, and physical stresses and in research studies are exposed to stresses associated with experimental protocols. Both populations suffer increased incidence of SDS. These observations have correlates to human SUDEP as stress increases seizure probability and SUDEP is more common in patients with uncontrolled epilepsy. [9]

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