

Perspective

The Standard of Perfection: Thoughts about the Laying Hen Model of Ovarian Cancer

Perspective on Hakim et al., p. 114

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In a way not possible in humans, cancer prevention scientists have used animal models to study the role of genes, molecular pathways and networks, and environmental factors that relate to carcinogenesis. These studies are also designed to qualify the animal model as an eventual source of preclinical evidence that will justify the introduction of promising cancer prevention agents into clinical trials. One such model, the white leghorn hen (a strain of *Gallus domesticus* bred for high egg production), has received several decades of attention for its unique attributes and scientific promise for exploring the etiology and prevention of epithelial ovarian cancer.

Early reports about ovarian tumor incidence in the white leghorn chicken showed that laying hens are subject to the spontaneous development of ovarian and oviductal adenocarcinomas, which are related to the extended maintenance of egg production in laying flocks (1). This observation is consistent with the hypothesis that incessant ovulation contributes to ovarian cancer risk in the laying hen model, just as it is thought to increase the risk of ovarian cancer in humans. Important features shared by hens and women include extended periods of repetitive epithelial injury and repair with associated inflammatory factors in a hormonal milieu. Clear advantages of the hen model compared with a typical animal model include spontaneous tumor formation without the need for an exogenous carcinogen or genetic engineering. Contrary to the assumption that domestication and selection for egg production has resulted in a highly inbred strain, analysis of genetic variation in several domestic lines of chickens, including the laying hen, reveal that genetic diversity in domestic chickens is similar to that of the wild chicken (*Gallus gallus*; ref. 2).

Ovarian cancer in the hen model has key similarities to ovarian cancer in humans. Little is known about the cell of origin or premalignant lesions, but in both species, ovarian adenocarcinoma is hypothesized to arise in cells derived from the ovarian epithelium, which in turn originates from the coelomic epithelium (3). In the hen model, the earliest recognized morphologic lesions are acini formed by a single layer of cells surrounding a lumen (1). As the tumor enlarges, the lumen may become slit like or filled by cribriform structures, and the tumor rests are often surrounded by dense fibrous connective tissue. In advanced disease, the abdomen is the predominant site of tumor activity, with serosal surface involvement that may lead to a

contraction of the mesentery, encasement of bowel, and production of ascites. When advanced tumors are disseminated in the abdominal cavity of a chicken, it is difficult to distinguish ovarian adenocarcinoma from oviductal adenocarcinoma, a second, less common form of genital tumor in chickens. This description of histology and morphology of tumors in the hen has striking parallels with human tumors, especially the challenge of distinguishing between advanced cases of fallopian tube carcinoma (the counterpart of chicken oviductal adenocarcinoma) and epithelial ovarian cancer (4).

Despite the similarities of ovarian carcinogenesis between chickens and humans, doubt remains as to whether chickens and humans are sufficiently biologically concordant to allow the identification of responses to potential interventions in the chicken that would be clinically meaningful for humans. The chicken is a fascinating vertebrate that has journeyed to the present from an evolutionary territory between reptiles and primates, an origin that distances the chicken from human comparators, in contrast to rodent models, which at least share some fundamental mammalian characteristics with humans. As in humans, the gonadal sex of the chicken is genetically determined at fertilization, but the female chicken has heterologous sex chromosomes. Gonadogenesis in the hen is strikingly asymmetric, leading to a single ovary on the left, with a regressed gonadal remnant on the right. With the onset of an ovulation-oviposition cycle in the chicken, follicular rupture occurs every 25 to 28 h with transit of the egg through the oviduct to the segment where albumin is added (magnum; ref. 5) and then on to the shell gland (uterus; ref. 6).

Comparing the molecular biology of ovarian cancer in chickens with that in humans generally has proceeded in small, focused studies. CA125 is a widely used marker for human ovarian cancer, but its functional significance is not fully understood. A recently reported immunohistochemical analysis (involving a high-temperature antigen retrieval method) found that CA125 expression was present in ovarian cancer cells from chickens but not in normal chicken ovaries (7). Another study looked at the expression of vascular endothelial growth factor (VEGF) in ascites cells and fluid from chickens with ovarian cancer (8). As in humans, the ascites volume seemed to be correlated with VEGF expression. In the current arena of molecular biology, the selective comparison of similarities and differences between ovarian tumors in humans and those in chickens affords an opportunity to further understand the pathogenesis of ovarian cancer, and many such opportunities are possible. Based on using this approach, a study reported by Hakim and colleagues in this issue of the journal (9) analyzed selected genetic alterations in ovarian and oviductal adenocarcinomas of the chicken.

These investigators evaluated tumor samples from a two-year chemopreventive intervention study in two flocks of white leghorn chickens. Before study initiation, the flocks ovulated

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almost daily for approximately 2 years. During the study, ovulation in flock A was suppressed by calorie restriction and reduced light exposure. Continuing with usual caloric intake and light exposure, ovulation persisted nearly daily in flock B. Genetic characteristics of ovarian tumors from the hens did not vary according to chemopreventive agent exposure. Ovarian adenocarcinomas were examined from 102 hens in flock A and from 70 hens in flock B, providing the main finding that p53 abnormalities were highly associated with 4 years of ovulation (96%, or 67 of 70 hens) versus 2 years of ovulation (14%, or 14 of 102 hens). In these tumors, K-ras mutations were uncommon (2 in 172 birds). Overexpression of HER2/*neu* by immunohistochemistry was tested only in chickens from flock A, in which testing of all 17 oviductal tumors and a subset of the ovarian adenocarcinomas was done. HER2/*neu* was overexpressed in only one of the oviductal tumors and in 53% of 19 ovarian adenocarcinomas. The pattern of p53 mutation seemed to differ according to duration of ovulation in that deletion mutations occurred in the DNA-binding domain of p53 in the small number of tumors from flock A, but missense mutations in the proline-rich domain were frequently identified in the tumors from flock B. The authors note that interspecies differences make it difficult to relate specific p53 mutations in chicken tumors to information about mutations in human ovarian tumors. Nevertheless, this study presents an extensive evaluation of pathologic specimens from the ovaries and oviducts of chickens, confirming an overall cross-species similarity in patterns of expression for some key genes associated with ovarian carcinogenesis, particularly p53. The authors plan a separate paper on the chemopreventive interventions complementing the genetic analysis reported here.

A current working hypothesis for ovarian cancer pathogenesis suggests that epithelial ovarian cancer in humans diverges along two broad lines of development, with abnormal p53 functional status serving as a distinguishing feature (10, 11). The p53 pathway remains intact for most tumors of low malignant potential. In this group of tumors (type I), a K-ras mutation is found in ~60% of borderline tumors and in about 70% of low-grade tumors. In contrast, K-ras mutations are not found in high-grade tumors, but mutations in p53 are observed in as many as 80% of the aggressive cancers (type II). In view of this binary classification, the results from Hakim et al. suggest that adenocarcinomas in the laying hen model have an important commonality with the molecular profile of high-grade ovarian tumors in humans.

The occasion of this observation provides an opportunity to brainstorm the scientific future of the laying hen model, as well as to appreciate the remarkable history of the white leghorn chicken. In the 1930s and 1940s, my grandmother, who prized her Barred Plymouth Rocks, would have used a reference book entitled the *American Standard of Perfection* for any question about attributes that constituted an ideal chicken (12). If my grandmother could see the way chicken specifications have changed in only two generations, she would be amazed. The wild red jungle chicken, *Gallus gallus*, was the first nonmammalian amniote (a vertebrate with an amnion) to have its genome sequenced (13). Benefiting from this information and the recognition that the chicken is an important model organism for the study of developmental biology, studies of functional genomics in chickens are now supported by a GeneChip Chicken Genome Array from Affymetrix with 32,773 transcripts representing over 28,000 chicken genes

(14). Given the availability of this platform and associated resources, it is not unreasonable to expect that comprehensive molecular profiling of chicken tumors is within reach.

A survey of the state of the science of animal models suggests that there are advanced approaches to model validation that might be applied to the white leghorn chicken. One noteworthy example is an interspecies comparison of conserved gene expression features between thirteen mouse models of breast cancer and human breast tumors (15). This comparison is facilitated by an established classification of human breast cancers (16, 17), the capability to profile a collection of tumors from various murine models, and the resources to simultaneously classify tumors across species by computational technologies such as the significance of analysis of microarray and gene set enrichment analysis. In the prototype overview of mouse models, the results suggest that at least rodent models of breast cancer can be genetically sorted for a high versus low probability of faithfully replicating the biology of specific subtypes of human breast cancer in a clinically significant way. Will such an approach be able to identify a "standard of perfection" (to borrow a phrase from animal husbandry) for future developmental studies involving the white leghorn hen model? Until this question is answered, other options are available for investigating the utility of this model.

One such option is to address important gaps in the basic science of ovarian cancer development, which include missing information about the specific molecular alterations in ovarian tissues that define the early natural history of the disease. For example, p53 has been investigated in morphologically benign serous lesions of the fallopian tube and may be associated with human premalignancy (18). This and other potentially important molecular markers and signatures might be more easily investigated in avian samples. Another long-standing question is the clinical significance of heterogeneity in phenotypes such as oviductal and ovarian adenocarcinomas in both humans and the chicken, which arise under similar conditions of risk and may share risk-reducing scenarios. The value of the laying hen model may not derive from its literal translation to the human setting but from the identification of mechanistic factors that both resemble and differ from those in humans.

Another important question is whether preventive interventions in white leghorn chickens can accelerate access to preventive agents for humans at a high risk of ovarian cancer. Potentially effective agents identified in the hen model could be tested in relatively small prevention proof-of-principle studies in humans (e.g., in *BRCA* mutation carriers identified as candidates for risk-reducing bilateral salpingo-oophorectomy). The limited evidence gathered to date from completed intervention studies in animal models is not the main rationale supporting prevention studies in humans (19, 20).

The best lead for ovarian cancer risk reduction in humans may be the data from observational studies suggesting a protective effect related to oral contraceptives. A meta-analysis of such data estimated that women may experience a 15% to 30% reduction of ovarian cancer risk for every 5 years of oral contraceptive use, depending on the elapsed time since last use (21). Although a comparison of ovarian surface epithelium between women randomized to oral contraceptive or progestin and women randomized to placebo is unlikely, an alternative source of biomarker data is tissue from contraceptive studies in non-human primates. Because of similarities in reproductive and hormonal biology

between humans and the macaque or rhesus monkey (*Macaca mulatta*), ovarian samples from an oral contraceptive study in macaques have been useful for correlative studies of biomarker changes in the ovarian surface epithelium in response to a combined oral contraceptive and to levonorgestrel as a single agent (22). Oral progestin was strongly associated with modulation of transforming growth factor (TGF)- β family members in conjunction with an increase in apoptosis. A similar increase of apoptosis in the ovarian surface epithelium following administrations of an oral contraceptive to rhesus macaques has been observed in a second biomarker study (23). The results of these two macaque studies are useful for suggesting an attractive mechanistic explanation for a possible preventive effect of progestin on the ovarian surface epithelium in humans. Human epidemiology and macaque biomarker results provide a strong rationale for the design of prevention studies in animal models of ovarian cancer development using a risk-reducing progestin. Although Hakim and co-authors cite more than 20 reports of animal models of ovarian cancer development (9), the laying hen model is of particular interest for the reasons mentioned already.

In view of the preceding human and simian data, it is not surprising that two leghorn chicken studies have included an approach related to oral contraception. In a small pilot study of an injectable progestin (20), a small reduction in tumors was overshadowed by an unexpectedly low tumor incidence, which may have been related to case ascertainment or to periodic suppression of egg laying by scheduled withdrawal of food and light to simulate the cessation of egg laying that would occur with the practice of "molting" under nonexperimental conditions (24). In an abstract (19) related to the present study by Hakim et al. (9), Rodriguez and colleagues reported preliminary results from a study in 2,400 2-year-old birds that suggest a reduction in gonadal tumors related to the inhibition of ovulation by caloric restric-

tion and a further decrease associated with progestin. Discovery work by this group of investigators has suggested that vitamin D alone (which has apoptotic effects mediated by TGF- β) may reduce ovarian cancer and enhance the preventive effect of progestin in the hen model (ref. 25; personal communication, Rodriguez GC, January 6, 2009), providing evidence in support of testing a vitamin D plus progestin combination in early-phase clinical trials. Because chicken tumors seem closely tied to the number of ovulation events and because the magnitude of reduction in relative risk associated with oral contraceptives in women may exceed the benefit that could be expected strictly based on a reduced number of ovulatory cycles (26), there are obvious questions about how the results in chickens relate to the observational oral contraceptive results in women. Nevertheless, the similar cross-species progestin results support the applicability of the hen model to ovarian cancer prevention in humans. A better understanding of the similarities and differences between hens and humans should be useful.

The elusive standard of perfection may be most relevant when it directs us toward a test or intervention that saves time and resources in the quest for a clinically meaningful result such as ovarian cancer risk reduction. It is not clear that the white leghorn hen model can achieve such a standard, but with the work of investigators like Hakim and colleagues, it can be hoped that the growing weight of evidence will help to move science in the right direction.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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