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One Medicine: An Introduction

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All of nature has inherent value for biology. Consider how much we have already learned from the comparative biology of yeast, worms, flies, zebra fish and many other diverse organisms about the molecular machinery governing normal cell division and normal growth and development; knowledge that applies also to mammals. Plant systems have advanced our understanding of epigenetics that might also apply to cancer biology. Aberrations in these basic processes such as the cell cycle check points and chromosome alignment on the mitotic spindle contribute, of course, to cancer. Naturally occurring oncogenic retroviruses associated with tumors in animals – mainly domesticated chickens, mice and cats, gave us our first glimpse, about 30 years ago, of oncogenes, cellular genes highly conserved throughout mammalian evolution. I experienced the excitement of this era by helping to discover two oncogenes, both cytoplasmic tyrosine kinases (see below) in spontaneous sarcomas of domestic cats. In studying retroviruses in wild mice as a potential model for human cancer, I also learned the importance of natural history knowledge in contrast to the information gained from laboratory mice. In the 1980s, DNA tumor viruses in animals pointed the way to the key role of evolutionarily conserved tumor suppressor genes with which the viruses interacted and inactivated. In the modern genomic era, we now appreciate that all life forms, and mammals in particular, have evolutionarily related genomes and are governed by similar fundamental molecular processes.

We know that oncogenes and tumor suppressor genes, for example, are normal cell genes that function in an intricate network together with many other genes, making up more than 30 signal transduction pathways and protein interaction domains that govern normal cell growth, differentiation and death (apoptosis). Hundreds of oncogenes and tumor suppressor genes and their protein products have already been identified as part of these pathways and domains. Point mutations or altered expression of these genes caused by chromosomal structural abnormalities may contribute to cancer in general and can be detected by modern techniques of cytogenetics, functional genomics and proteomics which allow the near simultaneous analyses of thousands of genes at the RNA and protein levels. Addition to or removal of phosphorous from proteins via protein kinases and phosphatases, respectively, is a key regulator of normal protein interactions in the signal transduction pathways and key driver of many forms of cancer. Immunoassays now permit the detection and quantitation of such intracellular factors and their extent of phosphorylation. Over 500 separate protein kinase genes are encoded in the mammalian genome, but relatively few of their targets are known. However, a good number of these kinases have already been linked to the development of cancer in general and as specific targets of therapeutic intervention. Other genes and proteins involved in signal transduction pathways, such as those controlling chromatin structure, telomere length, RNA transcription, RNA interference, protein folding and stability, cytoskelatal regulation, cell motility and angiogenesis are also highly conserved in evolution. Single nucleotide polymorphisms or larger structural variation in such genes could help explain the familial predisposition to certain cancers. Many other

gene variants and related proteins await discovery, not only in respect to human cancer but also from conserved orthologs contributing to cancer in other mammals. At the epigenetic level, the last decade has uncovered a "new genomics" in which different kinds of small RNA molecules, e.g. microRNA, regulate or inhibit gene expression at the post-transcriptional level. Thus, the concept of "one medicine" is our gospel and we realize the value of comparative pathology and molecular pathogenesis in furthering our understanding of the multihit kinetics of cancer and other diseases.

Breast cancer in humans and other mammals probably arises similarly from a single progenitor or stem cell, yet to be positively identified, hypothesized to reside in the epithelial lining of the terminal ductules of the lobular-alveolar unit. Such stem cells, under hormonal control, are thought to be responsible for repopulating the lining epithelium of the terminal ductules and larger ducts and for forming the lobular acinar cells that secrete milk before undergoing cell death or apoptosis. A putative mammary stem cell in the mouse has been able to give rise to an entire breast ductular system in vitro. The molecular pathways that govern this reversible normal process of stem cell renewal, cellular differentiation and involution are just now being identified; a master regulator gene has been identified in humans whose function could well underpin the normal behavior of stem cells as well as the initiation and early stages of breast cancer. Environmental risk factors e.g. diet, obesity, hormone pills, etc. impact these pathways primarily by their effect on the hormonal milieu, in particular by causing an unremitting exposure of the precursor stem cells to estrogens. Inherited tumor suppressor gene mutations are responsible for only 5–10% of human breast cancers; thus environmental factors and aging appear most important in triggering the critical mutations and epigenetic events such as DNA methylation and histone modification. Once the progenitor cell in the terminal duct epithelium acquires a critical number of mutations that activate oncogenes or inactivate tumor suppressor genes, together with epigenetic changes affecting gene regulation, the cell begins to grow out of control and its progeny become increasingly independent in their growth regulation as they acquire additional mutations – a process called clonal evolution. The neoplastic cells multiply within the lumina of the terminal ductules where they may become sufficiently aberrant in morphology to be called "carcinoma in situ" by the pathologist. This lesion is probably the precursor to invasive breast cancer in all mammals. Screening by mammography has greatly increased the detection of precancerous carcinoma in situ and such lesions have been harvested by core biopsies for pathology, gene expansion profiling and proteomics. These analyses have shown that most of the critical or "driver mutations" underlying invasive breast cancer in humans are already present in the precursor carcinoma in situ cells. However, clinically, after many years, only a small fraction of such carcinomas in situ actually become invasive cancers. The "holy grail" of breast cancer research is now to define the critical mutations, structural variations, or signature genes and affected molecular pathways that cause the transition from normal to benign carcinoma in situ to malignant invasive breast cancer.

Abnormalities in many genes and their products have already been found to be involved in the molecular pathogenesis of breast cancer in humans. At least 30 or more oncogenes and even more tumor suppressor genes and their associated molecular pathways are linked to breast cancer in humans. No two breast tumors have the same genetic profile but certain genetic themes or signatures and their associated protein pathways are coregulated and often repeated in various combinations. Among the oncogenes are, as expected, the estrogen and progesterone receptors and other growth factor receptors e.g., epidermal growth factor receptor (EGFR), whose activation promotes proliferation of the mammary epithelium. Some of these genes and associated pathways, e.g. estrogen receptor, have been linked to early stages of breast cancer, others such as EGFR more often have prognostic value, i.e., recurrence and progression of primary breast cancer. Gene expression assays, based on microarray technology, are being done on breast tumor samples to estimate the risks of recurrences and metastasis and probability of benefits from certain chemotherapeutic treatments, the basis of "individualized medicine". Effective treatment aimed at estrogen receptors, EGFR and certain protein kinases and angiogenesis are now used to treat breast cancer and prevent recurrence. In many instances, the results have been dramatic with a marked decline in early recurrence and reduced mortality.

Distinguishing "driver mutations" responsible for irregulated early growth of tumors from background noise of "passanger mutations," occurring in more advanced tumors but not contributing to their growth, is now being attempted using global DNA analysis and statistical interpretation for selected vs irrelevant mutations in potential oncogenes such as tyrosine kinases. If driver mutations and their altered pathways are identifiable, targeted therapy might be developed to stop or at

least slow down the progression of breast cancer in its earliest phase, obviously the beast chance for a "cure". The paradigm for this possibility is, of course, the effective treatment of chronic myelogenous leukemia by drugs that inhibit the unique and specific oncogenic protein. The dilemma we face, however, with breast cancer and most other cancers is that the critical mutations are usually multiple and affect multiple signal transduction pathways. Furthermore, additional mutations usually allow the initial key mutations to escape the inhibitory effect of the targeted therapy.

Oncogenic proteins, circulating in the blood stream and antibodies to these products can serve as potential biomarkers for the early detection of breast cancer or monitoring the response to therapy. The host immune response including fibrous tissue proliferation is undoubtedly involved in the generally futile effort to control the invasive cancer. It is even possible that the inflammatory reaction may contribute to the cancer progression. Accordingly, current studies aim to examine the molecular crosstalk that helps to explain these interactions of breast cancer cells with host defense mechanisms.

Historically, for over a century, spontaneous breast tumors in inbred mice were the model for breast cancer in women. Discovery of the murine mammary tumor virus (MMTV) in the 1930s prompted a fruitless search for a similar virus in humans but led, in the 1980s, to the identification of certain cellular oncogenes activated by the virus. Interestingly, MMTV is ubiquitous in aboriginal (wild), outbred mice, yet breast cancer is a rare occurrence, a finding that highlights the vital contribution of inbreeding to the genetic susceptibility to breast cancer in laboratory mice. The histologic lobular pattern of the MMTV-induced murine breast tumor was quite distinct, however, from the common histologic pattern of invasive ductular breast cancer in humans. With the introduction, about 20 years ago, of genetically engineered mice (GEM), it became possible to test the tumorigenic effect of different genes in transgenic mice. The same oncogenes that were naturally activated by MMTV induced the same kind of tumor when inserted with the MMTV promoter into germ cells of transgenic mice. But remarkably, when other oncogenes derived from DNA tumor viruses, or from human breast cancer, were similarly inserted with the MMTV promoter into mouse germ cells, the tumors that developed in the transgenic mice were often virtually indistinguishable histologically from the common infiltrating ductular breast cancers seen in humans. As first pointed out by Robert Cardiff, our Editor, the phenotype of the breast tumor often but not always predicts its genotype and vice versa. To me, this observation seems like magic, almost too good to be true. The results heralded the marvelous potential of GEM technology, which is still being fulfilled two decades later with over 100 breast cancer models under study in GEM mice.

As models for breast cancer, GEM surely deserve the limelight. Oncogenes or tumor suppressor genes known or suspected to be involved in human breast cancer can be inserted or depleted, respectively, in GEM to see if these manifestations induce similar or different appearing tumors. Many other genes, from human or animal, whose oncogenic function is uncertain, can be tested or validated for their function in GEM. The ability in GEM to turn the transgenes on or off at specific times and in specific locations, via tissue specific promotors, provides a marvelous opportunity to decipher the molecular pathways and their crosstalk involved in tumorigenesis and to discover new targets for therapy. A great advantage of the mouse model, not possible in any other animals, is the well proven transplantation test in cleared mammary fat pad for determining preneoplastic, i.e. carcinomas in situ, vs benign hyperplastic intraductal cell proliferation. Benign preneoplastic cells will grow, but not metastasize, in the fat pads but will not grow at all as subcutaneous implants. Truly malignant cells will grow autonomously in both locations and may metastasize. Of course, GEM have their limitations and, as man-made artifacts, can not be expected to absolutely recapitulate the biology of human breast cancer. For example, in transgenic mice, every cell and not just the stem cell carries the transgene, the host response is quite limited in terms of inflammation and fibrosis and the pattern of metastases is quite different from that of human breast cancer. Alexander Borowsky will cover the major attributes of GEM and their contribution to our current understanding of breast cancer.

For many years, the chemical and hormonal induction of breast tumors in laboratory rats has been well utilized for the induction of estrogen sensitive breast cancers. Many of these tumors are benign fibrodenomas rather than invasive cancers. Nevertheless, modern analysis now allows a deeper understanding of the interaction of environmental chemicals with genomic DNA in the causation of breast tumors in this rat model. DNA profiles of mutated breast cancer genes can even discern the causative chemical carcinogen in this model. Numerous chemoprevention strategies to prevent chemically induced breast cancer e.g. soy in the diet, have also been tested in this system. Michael Gould,

James Shull, Dan Medina and Helmut Zarbl discuss various aspects of this model system.

Domestic companion animals represent a rich source, largely untapped, for studying the comparative pathogenesis of naturally occurring breast cancer. Related genes, shuffled about on different chromosomes than in humans, are almost certainly involved. Comparative analysis of mammalian genomes indicates that most of the evolutionarily conserved sequences do not represent protein-coding genes but rather are noncoding elements important in gene regulation. Other lineage specific genes could, of course, contribute. With their genomes now completely sequenced the potential value of better understanding the natural history of these animal models seems immense for comparative pathogenesis study. Foremost are dogs, then cats. Breast tumors are even more common in dogs than in women. Somewhat like the rat, many such tumors are benign fibroadenomas or "mixed tumors", some of which become malignant. Other tumors are cancerous from the onset. Additionally, multiple tumors of both types can occur in the same animal. As mentioned before, all of these tumors probably arise from the same progenitor cells in the terminal ductules, are hormone responsive in their early stages, and involve similar genes or orthologs of genes implicated in human breast cancer. Because the same animal may harbor normal breast tissue as well as benign and malignant breast tumors and can be followed prospectively, analysis of global gene expression and protein interactions from these different tissues at different time points could provide insight into the critical genes and pathways involved in the progression of normal to benign to malignant neoplasia of breast epithelial cells. In addition, this comprehensive analysis can reveal "crosstalk" that leads to proliferation of myoepithelial cells and other mesenchynal cells that make up the "mixed tumors". Such tumors also offer the possibility of studying circulating biomarkers, antitumor immune responses and the contribution of host inflammatory cells and their products to the neoplastic process. Candidate cancer genes isolated from canine tumors can be validated for their function in transgenic mice. Homologs of such genes might also, of course, be involved in human breast cancer, remembering that we have only identified a small fraction of the full repertoire of 350 or so oncogenes thought to play a role in the generality of human breast cancer. Selective breeding has created a predilection for breast tumors in certain canine breeds and these tumors could provide a rich source of inherited inactivated tumor suppression genes. Some of these genes, as yet, undiscovered, could also have their function validated in "knockout" GEM and their possible role in human breast cancer determined. This new knowledge could, of course, also benefit the treatment of dogs with cancer.

Breast tumors in domestic cats are common enough and constitute a valuable model for poorly differentiated estrogen non-responsive breast cancers in women. The host inflammatory response and tumor cell metastatic pattern, including bone, are similar to humans. As in dogs, cats with breast cancer have normal breast tissue and sometimes precancerous hyperplasic lesions; these features enable informative comparison of global gene expression profiles. Many of the same oncogenes and tumor suppressor genes involved in human breast cancer are already known to be involved in canine and feline breast cancer. New genes await their discovery, counterparts of which are certainly involved in human breast cancer. Paraffin embedded tissue sections from hundreds of dog and cat breast tumors are available for PCR analysis of DNA mutations and immunohistochemical staining for levels of gene expression. Because dogs and cats share our environment, their tissues could also serve as a repository for potential carcinogens such as insecticides that have been suspected as etiologic agents in human breast cancer. Dogs and cats also live much longer than rodents, an advantage for observing the multihit kinetics of breast cancer onset and progression.

Captive macaques, many thousands in number in primate facilities, offer another potential model. The macaque genome is now completely sequenced, and conserved genes are 93% homologous in sequences to humans. Although not frequent, precancerous lesions and invasive breast cancer indistinguishable from those in humans have been seen in a modest number (lifetime incidence approximately 6%) of aging macaques at different primate centers. Macaques given various hormones have shown uprequlation of proliferation markers in mammary ductile epithelia as well as alterations in estrogen and progesterone receptors, oncogene receptors and the development of preneoplastic changes. This valuable nonhuman primate resource begs to be exploited by our modern techniques to delve into the molecular pathogenesis of breast cancer. Similarities with human breast cancer are a certainty. Macaques with a familial predisposition to breast cancer could be inbred, given hormones, allowed to age and subjected to mammography screening for detecting carcinoma in situ. One wonders whether macaques could be inbred to the extent that they would accommodate transplant of

precancerous lesions into their cleared mammary gland fat pad as has been done so successfully in mice. Only in this way can we determine the true preneoplastic nature of the in situ carcinomas observed in this model.

Finally, think for a moment about the almost total absence of breast cancer in cows, an animal whose mammary glands represent the maximum in structure and function. It could certainly be informative to analyze global patterns of gene expression and protein interaction domains in the terminal ductular epithelium and its progenitor stem cells that repetitively differentiate, secrete and then involute, all under hormonal control. The expression and localization of estrogen and progesterone receptors in the bovine mamm–ary gland during development, lactation and involution has been described using commercially available anti-human antibodies. Understanding better these fundamental molecular pathways and their regulation could give valuable insight into the key "players", whose counterparts, when altered, could initiate and promote breast cancer in humans.

In so far as the breast cancer epidemic in humans remains untamed, it behooves us to learn as much as possible as quickly as possible about the molecular pathogenesis and genetic complexity of breast cancer in all of nature's examples. We can all benefit from the practice of "one medicine."