



## Mouse models for breast cancer

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Fifteen years ago, Philip Leder and his colleagues generated a transgenic mouse that developed mammary tumors as a result of the expression of the human oncogene *myc* in the mammary epithelium (Stewart *et al.*, 1984). Three years later the same group demonstrated the synergism between different oncoproteins, and thus set the stage for the cooperative and multi-event cancer model (Sinn *et al.*, 1987). These classical papers are milestones in breast cancer research, and they set into motion an ever-accelerating research train poised to explore genetic pathways in breast cancer. Federal and private funding agencies alike saw an opportunity to promote efforts aimed to identify and understand genetic pathways in breast cancer in settings resembling the human condition. A recent study by Xu, and her colleagues (Xu *et al.*, 1999) exemplifies another milestone towards this goal. They succeeded in the inactivation of the breast cancer gene *Bracl* specifically in mammary epithelial cells of mice, and demonstrated that these mice developed mammary tumors coinciding with genome instability (see Deng and Scott, this issue).

Understanding genetic pathways was considered to be, and still remains, the prerequisite for the development of molecular and pharmacological therapeutics to treat and prevent cancer. Over the past 15 years the use of transgenic and gene deletion mice in mammary research has been documented in more than 100 scientific articles. These studies have provided an in-depth insight into molecular pathways of both normal mammary development and tumorigenesis. While studies in tissue culture cells permit the molecular dissection of pathways operative in a single cell, research in mice integrates the complexity of an organ and its different cell types with the dynamic hormonal and physiological status of the animal. One important lesson from these studies points to an equally important role of the stroma in the development of the mammary gland (Hennighausen and Robinson, 1998).

The impact of transgenic mouse models on breast cancer research was the topic of a recent conference in Annapolis. This conference (March 3–5, 1999) was sponsored by the National Institutes of Health. More information can be obtained at <http://mammary.nih.gov/Annapolis-guidelines>. It is clear that not a single

mouse model covers the full spectrum of the human disease. Rather, researchers have now the luxury to pick any particular genetically modified mouse to study specific signaling pathways, physiological events, hormonal contributions and therapeutics. At the Annapolis conference, pathologists and basic researchers gathered to discuss and evaluate the biology and pathology of mouse models in the framework of the human condition. A panel of nine medical and veterinary pathologists with expertise in mammary gland biology reviewed material representing more than 90% of the mouse models. A nomenclature was developed and recommendations for future analyses were drafted. The consensus report from the Annapolis meeting, including the 'Annapolis guidelines' is published in this issue of *Oncogene* (Cardiff *et al.*). The Internet offers the opportunity to disseminate complex biological and histological information. A web based interactive histology atlas was developed (Evans *et al.* this issue) that facilitates the comparison of high resolution images from mouse models and human breast cancer (<http://histology.nih.gov>). Sophisticated software permits researchers sitting in different locations to view, discuss, annotate and compare histological images. This, in conjunction with biological databases (<http://mammary.nih.gov>) will usher in a new era for the digital analysis of mouse models.

In assessing the validity of mouse models for cancer research it is necessary to consider several parameters. These include the nature of the oncogenic stimulus, the promoter used to target transgene expression, the integrity and status of endogenous signaling pathways, the spectrum of additional mutations that arise, the genetic background of the mouse strain, and of course the molecular pathology and histology. Reviews in this issue of *Oncogene* address these aspects and provide the rationale for an in-depth evaluation of transgenic mouse models for human breast cancer. The first inbred mouse strain having a high incidence of mammary tumors was developed more than 60 years ago at the Jackson Laboratory in Bar Harbor, Maine, USA. Bittner's demonstration in 1936 of the 'milk factor' in mouse strains having a high incidence of mammary tumors led to the discovery of the mouse mammary tumor virus (MMTV) and cellular proto-oncogenes that are activated by juxtaposed MMTV proviruses. Callahan and Smith (this issue) present a historical perspective and discuss the genetic pathways that were discovered through MMTV induced mutagenesis. *Wnt-1* was the first gene discovered to be activated by a juxtaposed MMTV provirus and Li *et al.* (this issue) provide testimony on how that gene helped to identify and dissect molecular pathways in mammary tumorigenesis. The *wnt* transgenic mice were generated more than a decade ago and have been a unique resource (like the *myc* 'oncomice' from the

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Leder laboratory) geared towards understanding the interactions and cross talk of signaling pathways in tumor progression.

A variety of oncogenes and growth factors have been expressed in transgenic mice, and the respective DNA control elements probably influence features of the mouse model. In addition to the long terminal repeat of the mouse mammary tumor virus (MMTV-LTR), promoters from milk protein genes (WAP,  $\beta$ -lactoglobulin,  $\beta$ -casein) and the C3(1) promoter have been used to control transgenes. These control elements target the mammary epithelium, and they are stimulated by lactogenic hormones. It is worth keeping in mind that these promoters differ in their temporal and spatial activity, which certainly affects the outcome of an oncogenic stimulus.

While some genes, such as *myc*, *p53* and cyclins (Dickson *et al.*, this issue), provide an obvious link to human cancer, the use of others, such as viral oncogenes and *wnt* genes, is less direct. Viral oncogenes disrupt key nuclear and cytoplasmic signaling molecules operative during normal development, and the respective mouse models provide insight into pathways and should be used in their own right. Articles by the laboratories of Furth, Deppert, Green and Müller (this issue) discuss models in which cell cycle pathways are disrupted. The use of the SV40 T antigen, which binds to and inactivates the cell cycle regulators p53 and pRb, continues to provide mechanistic insight into cancer progression. The tumor suppressor gene p53 is mutated in approximately 50% of primary human breast cancers, and its role in carcinogenesis has been addressed directly in mouse models through the generation of specific mutations. The articles in this issue by Jerry and colleagues and Murphy and Rosen address the contribution of mutated p53 genes in tumor initiation and progression. Taken together these studies provide compelling evidence that both p53 dependent and p53 independent pathways are operative in mammary tissue.

While many studies in the past addressed the role of individual genes in tumorigenesis, more recently the interaction of oncoproteins with each other, with growth modulators, and with cell survival/death signals has been explored. Specifically the synergistic role of oncogenic signals with growth regulators, such as TGF $\alpha$

and *myc*, and cell survival signals, such as members of the *bcl-2* family, has been investigated. Articles in this issue by Jamerson *et al.*, Li *et al.*, Rose-Hellekant and Sandgren, and Murphy and Rosen address this issue. With the ability to delete genes from the mouse genome (generation of knock-out mice) it is now possible to reliably explore the interaction of oncogenic stimuli with endogenous signaling pathways. Li *et al.* and Green and his colleagues demonstrate the contribution of cell death molecules from the *bcl-2* family in tumor progression. The lactogenic hormone prolactin is critical for functional mammary gland development and its growth stimulatory activity has long been suspected to synergize with oncogenic stimuli. Vomachka and colleagues use prolactin knock-out mice to address this issue, Wennbo and Tornell analyse transgenic mice that overexpress prolactin, and Humphreys and Hennighausen use Stat5a-null mice to evaluate the role of the Jak/Stat pathway (which receives the prolactin signals and conveys it to the nucleus to initiate developmental programs).

A litmus test whether a mouse model bears relevance to the human condition is also based on the molecular pathology and histology. The pathologists at the recent Annapolis conference reviewed material from 33 mouse models and they developed and agreed upon a morphological nomenclature that allows the direct comparisons among models and across laboratories using uniform criteria (Cardiff *et al.*, this issue). It is suggested that the Annapolis nomenclature be adopted by the research community and in federally funded research. After 15 years of intensive and productive research the mammary community has identified genetic pathways of breast cancer, and therapeutic and preventative compounds are now being tested in mouse models (Bearss *et al.*, this issue). However, our understanding of pathways controlling normal mammary physiology in the mouse and human is still rudimentary, and we are only at the beginning of the road to replicate human cancer in mice. While researchers in the 20th century focused on the identification of signals and genetic pathways that control mammary development, the beginning of the 21st century will need to focus on the interphase of normal physiology and cancer.

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