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Precancer in Mice: Animal Models Used to Understand, Prevent, and Treat Human Precancers

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ABSTRACT

We present a status report from the NCI Mouse Models of Human Cancers Consortium (MMHCC) Precancers Workshop held November 8 and 9, 2004. An expert panel, the Mouse Models Group (MMG) evaluated the status of mouse models of precancer emphasizing genetically engineered mouse models, especially of lining epithelium and their utilitarian value to human carcinogenesis. An outline of the background for the panel's considerations is provided with examples of past and current precancerous lesions in mice. The experimental use of oncogenic viruses and chemical carcinogens in mice led to operational definitions of initiation, promotion, and preneoplasia Preneoplastic and precancerous lesions are found in these models. In this precancer concept, most preneoplastic lesions are considered as potentially precancerous or at least an earlier stage in cancer development than typical pre-invasive epithelial lesions, which are often seen in these mouse models. Genetically engineered mice, used to test the oncogenicity of individual genes, develop precancers that are initiated by defined molecular and histopathologic changes. The mouse can be used to isolate and study precancers in detail, thereby providing a level of biological understanding not readily available in clinical disease. These studies suggest that genetically engineered mice are very useful preclinical models for chemoprevention and therapy.

Keywords. Precancer; mouse; models; GEM (Genetic Engineered Mice); pathology; preclinical; trials.

INTRODUCTION

Potential precancers were recognized in laboratory mice as early as 1911 (Haaland, 1911) with the identification of the hyperplastic alveolar nodule (HAN) of the mammary gland. The term "precancer" first appeared in the English literature in 1914 with application to human breast cancers by Ewing (1914). Many of the key concepts of neoplastic progression, such as initiation and promotion, were developed using the mouse skin and have been reviewed (Foulds, 1958, 1959; Yuspa, 1994). Over the decades, potentially preneoplastic (precursor of benign tumors) and precancerous lesions have been identified and studied in almost every epithelial organ system (Foulds 1959, 1975). For this review of the precancer concept, we include both preneoplastic lesions and precancerous lesions especially in lining epithelium, as both "precancers," although in some tissues, such as liver, preneoplastic lesions may only lead to benign neoplasms; sometimes they lead to a low incidence of carcinomas. Historically, the morphological criteria for epithelial precancers have been focality and atypia in association with malignant tumors (Cardiff et al., 2000b). These are the Group B lesions of Foulds (Foulds, 1959) that were first described by Waldeyer in 1867 (Waldeyer, 1867; Rather, 1978). The lesions are expected to

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Abbreviations: ACF, aberrant crypt foci; BCAC, *B*-catenin-accumulated crypts; DMBA, 7, 12-dimethylbenz(a)anthracene; GEM, genetically engineered mouse; HAN, hyperplastic alveolar nodule; mGIN, mouse gastrointestinal neoplasia; MMG, Mouse Models Group; MMHCC, Mouse Models of Human Cancers Consortium; mMIN, mouse mammary intraepithelial neoplasia; MMTV, mouse mammary tumor virus; mPIN, murine prostatic intraepithelial neoplasia; mSkIN, mouse skin intraepithelial neoplasia; SENCAR, sensitivity to carcinogens; TPA, 12-O-tetradecanoylphorbol-13-acetate.

form a demonstrable morphological continuum culminating in malignancy (Park et al., 2002). The initial observation of potential premalignancy usually involves the identification of an association of foci of atypia with adjacent malignant neoplasias. Further substantiation is provided if the precancerous lesions appear before the emergence of the malignancy (Park et al., 2002). Such temporal association is reinforced by observation of the continuum from increased cellularity (hyperplasia) through cellular atypia (dysplasia) and atypical focal growths (preneoplasia) to malignancy. Increasingly abnormal morphology provides a logical continuum with increases in severity, coupling size and grade with "progression" culminating with a palpable discrete mass. This temporal element cannot be captured in tissues frozen in time for microscopic examination. In most cases, the antecedent lesions are more abundant than the malignant tumors, implying varying biological potential. The assessment of the biological potential of any single focus of atypia becomes hampered, because it has lost "potential" during removal and fixation.

In human biology, the study of precancers is compounded by the apparent biological heterogeneity of the lesions. Not all atypias result in cancer. In order to identify the higher-risk lesions, clinical scientists have resorted to epidemiological and statistical techniques to test and validate morphology-based hypotheses (Cardiff et al., 2004). This approach has substantiated the "association" between precancers and cancer sufficiently to stimulate surgical intervention in such conditions as familial polyposis or cervical intraepithelial neoplasia. The reduction in the incidence of these invasive diseases has led to dramatic biological proof by therapeutic trial. Such outcomes have encouraged explorations of potential precancers, which have not been as successful. The summaries of the Progress Review Group of the National Cancer Institute sound a clarion call for more research of the biology of these precancers, or "early malignancies" ⟨http//planning.cancer.gov/disease/plans.shtml#prg⟩.

Our understanding of neoplastic progression has been enhanced by study of the process in the laboratory mouse. Using inbred animal populations reduces genetic heterogeneity inherent in the outbred human population. The investigator can control the temporal element by sequential sampling of animals known to develop focal atypias. The biological potential of any given focus of atypia can be tested by identifying the lesion in situ, surgically isolating the lesion, and transplanting it into a syngeneic mouse. This "test-by-transplantation" provides an operational definition of the biological potential.

This report provides a review of the field that led to the consensus of the Mouse Models Group from the NCI Mouse Models of Human Cancers Consortium (MMHCC) Precancers Workshop held at George Washington University, November 8 and 9, 2004. The meeting organizers asked the Mouse Models Group (MMG) to evaluate the current status of mouse models by addressing the questions of validity, applicability, and value of the available models in understanding, treating, and preventing human precancers. A group of experts pondered the questions, considered reviews of morphological and biological characteristics of murine precancers in various organ systems, and now offer their evaluation.

Mouse Precancer Terminology

Cancer develops through a multistage process in mice, as it does in other species (Maronpot, 1999; Ward et al., 2000; Cardiff, 2001; Ward, 2002). The first serial-sacrifice experiments in mice were analyzed to elucidate the histopathogenesis of spontaneous and chemically induced tumorigenesis. More recently, studies in genetically engineered mice have identified similar multistage progression in most epithelial tissues, such as skin, lung, liver, prostate, mammary gland, gastrointestinal tract, and kidney. Historically, a variety of terms has been used for precancerous lesions, including focal hyperplasia, atypical hyperplasia, preneoplasia, protoneoplasia, carcinoma in situ, dysplasia, microadenoma, microcarcinoma, intraepithelial neoplasia, premalignancy, and nodular hyperplasia. Proliferative lesions, such as aberrant crypt foci, precede benign tumors (e.g., adenomas and papillomas) and carcinomas and include hyperplastic lesions in flat lining epithelium and benign adenomas and papillomas like those seen in mouse liver, intestine and skin. Unlike neoplasms in humans that are classified as benign, those in mice classified as "benign tumors," occurring either spontaneously or induced by various etiologic agents or genetic manipulation, can progress to malignant tumors.

The array of descriptive and conceptual terms that has been applied historically to early neoplastic lesions in the mouse has led to a confusing and inconsistent terminology. We, therefore, propose applying the term "precancer" to all early preinvasive neoplasms described here. In the mouse, precancer is a physical entity that can be detected as a morphologically atypical epithelial focus that precedes a malignant (invasive or metastatic) neoplasm. The term is consistent with the definition developed by the workshop (Berman et al., 2006) and included in the previous classifications (Berman and Henson, 2003a, 2003b), which may include theoretical entities that cannot currently be detected as morphological entities; it may exclude some mesenchymal neoplasms. Mouse precancer has been characterized, in epithelium, as a neoplasm that is clonal, immortal, and limited by a basement membrane (Cardiff et al., 2000b); it is characteristically associated with molecular, morphological, and biological progression, evidenced by a continuum of changes ending in malignancy (Cardiff et al., 2000b). Human precancers that have been characterized have similar characteristics creating a biological, morphological and molecular link between the mouse and human diseases (Burstein et al., 2004). Synonyms may be used if they are associated with historical precedence or organ specificity.

Molecular changes have been found in these various steps of cancer progression in mice (Ward, 2002). Patterns of gene expression in human cancers and mouse models of human cancer, but not precancerous lesions, have been compared and may provide a validation of the mouse models (Graeber and Sawyers, 2005).

The ontology developed by the NCI MMHCC Pathology Committee placed the precancers described above into the category of abnormal growth, as a "child" of neoplasia and a "sibling" of benign neoplasia and malignant neoplasia (Cardiff et al., 2000a).

Mouse-Model Systems of Precancer

The principles discussed here are based on an extensive literature. With the advent of genetic engineering, virtually every organ has been targeted with oncogenic transgenes or has had tumor suppressor genes silenced. Studies found in the literature have documented that almost all of the tumors arising in genetically engineered animals are associated with potentially premalignant foci. We offer several examples of organ-specific models of precancers to highlight specific aspects of the disease in mice.

The Prototypic Premalignancy: Mouse Mammary Intraepithelial Neoplasia (mMIN)

The mammary gland has provided one of the prototypic biological systems for the experimental study of the biology of epithelial precancers. First recognized in 1906, the hyperplastic alveolar nodule (HAN) was later, in 1911, associated with mouse mammary tumors (Haaland, 1911). Subsequently, the HAN was found to be induced by the mouse mammary tumor virus (MMTV) (Cardiff et al., 2002). Because they appear long before mammary tumors emerge, HANs have been used as a "nodulogenesis assay" for MMTV infectivity (Cardiff et al., 2002). Development of the "test-by-transplantation" method was accomplished by isolating and transplanting HANs into gland-cleared mammary fat pads. These studies provided the operational biological criteria for "preneoplasia," because the abnormal outgrowths of HANs proved to be clonal immortal tissues that can grow only in orthotopic locations (mammary fat pad), but not ectopic sites in which malignant tumors grow. Since tumors emerge as subpopulations from HANs, they and their outgrowths are "pre," "proto," or "early" neoplasms (Cardiff and Aguilar-Cordova, 1988). Each outgrowth displays a characteristic rate of malignant transformation and a characteristic metastatic rate that define the relative risk. Transplants of normal ductal tissue result in outgrowth of normal mammary trees that do not undergo malignant transformation and they subsequently senesce after 3 to 5 serial transplants.

Preneoplastic outgrowths of virus-infected, carcinogen-induced, and genetically engineered mammary gland have been generated and studied (Cardiff et al., 2002). All of these different sources of initiating oncogenes have proven to result in early premalignant tissue with a high risk of transformation into malignancy. Thus far, however, predictive or prognostic microscopic or molecular biomarkers have not been identified consistently in any of the numerous outgrowth lines. The suggestion has thus been made that the biological potential is pre-encoded in a progenitor cell that has yet to be identified (Maglione et al., 2004).

Although the principles of focal atypical lesions have been utilized as criteria for landmark studies of precancers in the human breast (Ewing, 1919; Wellings et al., 1975; Rather, 1978), the histology of virus-induced mouse precancers and their tumors rarely resembles that observed in the human breast. With the advent of the genetically engineered mouse (GEM), however, numerous potential precancers have been identified that bear a striking morphological resemblance to human ductal carcinoma in situ (DCIS) (Cardiff et al., 2002). Moreover, these mMINs are frequently created in mice with molecular abnormalities modeled after those found in humans (Cardiff et al., 2000a, 2000b). The biological potential of these precancers has been successfully tested using 1 tumor suppressor gene (*p53*) and 1 oncogenic transgene (*Polyoma Virus middle T*) (Maglione et al., 2001, Maglione, 2004; Namba et al., 2004). These studies have confirmed that mMIN are heterogeneous premalignant tissues composed of clonal, immortalized cell populations. The mouse mammary gland sets the standard by which the biology of all precancers can be evaluated.

Premalignancy Defines Initiation and Promotion: Mouse Skin Intraepithelial neoplasia (mSkIN)

Studies of rodent skin using topical application of chemical carcinogens have provided an experimentally tractable model of neoplastic progression. Initiated over 50 years ago, these investigations have proven that invasive squamous cell carcinomas arise within a subset of the precancerous squamous papillomas and establish a temporal and spatial relationship between the two. By varying the schedule and order of application of topical chemical carcinogens, investigators have established the principles of tumor initiation and promotion (Yuspa, 1994). Specific mouse strains, including the SENCAR (SENsitivity to CARcinogens), have been developed specifically to evaluate potential chemical carcinogens. Some agents, such as benzo(a)pyrene were found to be effective in inducing cutaneous tumors as single agents (complete carcinogenesis), while others, such as 7,12-dimethylbenz(a)anthracene (DMBA), were able to induce the first stages of neoplasia after a single application (initiator) but required multiple subsequent applications of a tumor promoter, such as 12-O-tetradecanoyl-phorbol-13-acetate (TPA), for efficient tumor progression to occur. Activating mutations in *H-ras* were found both in early- and later-stage lesions (Balmain et al., 1984). The evolution of histologic and molecular markers that occurs with squamous tumor progression has been extensively characterized and reviewed (Klein-Szanto et al., 1993).

Chemical models of cutaneous carcinogenesis are still widely used to determine the effects of specific genetic alterations on tumor formation and progression using genetically engineered mice. In late generation Terc-/- mice that possess short telomeres, precancerous squamous papillomas still occur at a reduced rate following topically applied DMBA/TPA, but progression to invasive carcinoma is prevented (Gonzalez-Suarez et al., 2000).

Genetically engineered mouse models of progression from precancerous lesions to squamous cell carcinoma have also been produced by transgenic expression of the human-papilloma-virus-type-16 early region in basal keratinocytes (Arbeit et al., 1994). In this model, tumor progression occurs in several stages that include hyperplasia, dysplasia, and invasive squamous cell carcinoma with varying grades of differentiation. Important observations of the relationship of angiogenesis to neoplasia have been derived in part from this model, including the appearance of neoplasia-associated angiogenesis before full malignant transformation (Holland, 2004).

Precancerous lesions are characteristic of 2 GEM models of malignant melanoma. Overexpression of the metabotropic glutamate receptor 1 (mGluR 1) in melanocytes results in intensely pigmented melanocytic lesions resembling human

epithelioid blue nevi (Pollock et al., 2003). A subset of these lesions undergoes malignant transformation with metastasis and loss of pigment production (Zhu et al., 1998). Expression of hepatocyte growth factor under the control of the metallothionein promoter results in melanoma in situ lesions following perinatal exposure to ultraviolet light (Noonan et al., 2001). Extensive upward migration of melanocytes within the epidermis is characteristic of these lesions, some of which progress to invasive and metastatic melanoma.

Precancer in Association with Malignancy: Mouse Colon Intraepithelial Neoplasia (mGIN/ACF)

Most mouse models of colonic and small intestinal cancer have atypical crypt foci that are found adjacent the cancer. These lesions have been inferentially associated with precancers, and few of them have undergone the classical test by transplantation.

One of the more intriguing examples and extensively studied lesions has been the aberrant crypt foci (ACF), initially reported in 1987, in the intestine of carcinogen-treated mice (Bird, 1987). They are typically viewed and counted using methylene blue staining of whole-mount preparations. Although ACF are now considered the earliest precursors of human colorectal cancer, they illustrate some of the limitations in assessing progression in the murine intestine. Aberrant crypt foci have also been observed in some types of GEM. Spontaneous development of ACF has been reported in $Apc^{min/+}$, $Apc^{\Delta 716/+}$, and $Apc^{1638N/+}$ mice (Boivin et al., 2003; Cheng and Lai, 2003; Mori et al., 2004). Interestingly, $Apc^{1638N/+}$ exhibit typical "classical" ACF, whereas $Apc^{min/+}$ and $Apc^{\Delta 716/+}$ mice do not, because the lesions are not elevated above the surrounding mucosa (Pretlow et al., 2003). The lesions in these mice, denoted ACF, are also referred to as "flat" lesions. The ACF in the Apc mutant mice are located in the cecum and colon; however, adenomas are primarily observed in the small intestine. Thus, development of ACF in these mice does not parallel the typical ACF-adenoma-carcinoma progression. Carcinomas are rare in these mice in comparison to the number of ACF. This discordance illustrates the hazards of using association with cancer as a criterion without biological proof.

Two general types of ACF have been identified in rodents that parallel those in humans: ACF with a hyperplastic raised phenotype and ACF with a flat dysplastic phenotype. The dysplastic ACF are considered to be more predictive of malignancy in both humans and mice and may be considered mGIN. Not only are the histological features of these two lesions distinct, but the molecular alterations also parallel their malignant potential. Many of the molecular changes observed in adenomas and carcinomas are seen in the dysplastic ACF, but not the hyperplastic ACF. One of the most common molecular changes is altered control of β-catenin, with reduction in membrane staining and an increase in cytoplasmic and nuclear staining. Other alterations include increases in carcinoembryonic antigen and mutations of *K-ras*, *APC*, and *p53* in the dysplastic ACF (Cheng and Lai, 2003 Mori et al., 2004). In addition, PCNA and Ki-67 staining is increased in the upper portions of the crypts, consistent with the increased cellular proliferation present in the ACF (Cheng and Lai, 2003; Mori et al., 2004).

Recently, additional lesions have been identified in rodents that may also constitute a precancerous change. In azoxymethane-treated rats and mice, β-catenin-accumulated crypts (BCAC) are observed in the colonic mucosa (Mori et al., 2004). Importantly, the regional location of BCACs is similar to that of the adenomas and carcinomas that develop in azoxymethane-treated rodents. These lesions are similar to the flat ACF observed in the $Apc^{min/+}$mice. Another lesion type, the mucin-depleted focus, has also been described. That the BCAC, flat ACF, and mucin-depleted foci are more relevant biomarkers of colon cancer than the classic ACF has been postulated, because they are more similar histologically to adenomas and carcinomas, their number and crypt multiplicities correlate with carcinogenesis, and they respond like adenomas and carcinomas to carcinogenic and chemopreventive agents (Green and Hudson, 2005; Paulsen et al., 2005). Further work is needed to investigate the differences amongst these lesions and to verify that they are mGIN.

Pancreas (mPanIN):. Pancreatic ductal adenocarcinoma ranks among the most lethalof human malignancies. Pancreatic intraepithelial neoplasms (PanIN) are the premallignant lesions for human ductal adenocarcimonas (Hruban et al., 2001). Similar lesions have been produced in genetically engineered mice (Hruban et al., 2006). To distinguish them from human preneoplastic lesions, mouse pancreatic intraepithelial neoplasms are referred to as mouse PanIN or mPanIN (Hruban et al., 2006). Like the human PanIN, mPanIN is characterized by atypical epithelial proliferations confined to the pancreatic ducts, involving small ducts (<1 mm), mPanIN are distinguished from acinar-ductal metaplasia in that they show no acinar differentiation (Hruban et al., 2006) The degree of cytologic and architectural atypia in these lesions are graded in parallel to human PanIN (mPanIN-1, mPanIN-2, and mPanIN-3) (Hruban et al., 2001).

Two mouse models that develop mPanIN have recently been developed (Aguirre et al., 2003; Hingorani et al., 2003). In brief, newly developed models of pancreatic cancer exhibit many of the features of human PanIN and ductal adenocarcimona. Molecular biological studies are required in all of the pancreatic cancer models to further characterize the relationship of the histologic changes with those in human pancreatic neoplasias.

Other Organs

Precancers have been described in numerous organs from genetic engineering studies and other experimental systems using mice. Some GEMs, have been extensively studied. While the brief reviews provided here highlight the strengths and weaknesses of mouse models, several other organs used in classical studies in mice deserve mention.

The histopathogenesis of liver cancer has been well described in experimental and spontaneous mouse models. Preneoplastic foci, also termed foci of cellular alteration, altered foci, focal hyperplasia, and foci, have been described in many studies of mouse hepatocarcinogenesis and may be subclassified into eosinophilic, basophilic, clear-cell, or mixed based upon staining characteristics (Harada et al., 1996). Preneoplastic foci can progress to "adenomas" within which the development of carcinoma suggests that they themselves are premalignant, thus, precancerous (Jang et al., 1992). *H-ras* mutations are common in spontaneous liver tumors in

wild-type laboratory mice and may be more or less frequent in treated mice, depending upon the specific hepatocarcinogen (Maronpot et al., 1995). *H-ras* mutations have also been identified in some preneoplastic foci, although few studies have been done (Maronpot et al., 1995). Mutations were reported in the murine *H-ras* gene in spontaneous hepatocellular carcinomas, but not in the human H-ras gene in foci or adenomas of human rasH2 transgenic mice (Hayashi et al., 1998).

Preleukemic and prelymphomatous conditions have not been well described in mice (Morse et al., 2002). The earliest stage of follicular B-cell lymphoma in mice often appears as focal splenic white-pulp hyperplasia that can be clonal, determined by gene rearrangements (Morse et al., 2002). Splenic marginal zone lymphomas arise from marginal zone hyperplasias (Ward et al., 1999). T-cell lymphomas most often arise from a thymic atypical hyperplastic lesion following a depletion of small lymphocytes from one thymic lobe (Dunnick et al., 1997). Many of the induced T-cell lymphomas in GEM arise from the thymus. The origin of B cell and other lymphomas in GEM is often not studied.

The GEM in Preclinical Trials

Genetically engineered mice have proven very useful in toxicological studies. The Tg.Ac mouse has perhaps, become the most widely used GEM model system (Humble et al., 2005) for testing carcinogens. The model involves initiation with *H-ras* with the ability to test promoters and to test intervention strategies in the precancerous stages.

Studies using GEM models expand the horizon beyond histological and genetic similarities and illustrate that the next exciting steps in GEM are being taken in biomarker discovery; different biomarkers help diagnose, prognosticate, and predict response to drugs. These contributions have been elegantly illustrated in the GEM mPIN and other mouse models of cancer.

Multiple lines of evidence, encompassing anatomic, phenotypic, and genetic data, support the relationship between prostatic intraepithelial neoplasia (PIN) and prostate adenocarcinoma in humans (Shappell et al., 2004) Genetically engineered mouse models of prostate cancer, for the most part, faithfully phenocopy progression from normal to PIN to cancer. The GEMs have proven invaluable by providing the missing piece of the puzzle, proof of progression, which cannot be feasibly or ethically studied in humans. Small, morphologically atypical foci, identifiable in younger animals, expand with age. Progressive nuclear and cytoplasmic changes in the epithelium have been described and graded in SV40Tag and PTEN related models, fulfilling the criteria of a morphological continuum related to invasive cancer (Park et al., 2002). Molecular changes have also been found in mPIN lesions (Shibata et al., 1996).

Two groups have innovatively used GEMs to predict the molecular signature of prostatic cancer in humans (Ellwood-Yen et al., 2003; Wang et al., 2003). The utility of the MYC and PTEN signatures in murine prostatic cancers to predict similar human subtypes is now recognized (Ellwood-Yen et al., 2003; Wang et al., 2003). Preclinical information that either PTEN loss or AKT overexpression sensitizes xenografted human tumors to mTOR inhibitors shows that treatment with RAD001, a mTOR inhibitor and rapamycin analog, can reverse the mPIN phenotype in AKT transgenic mice (Majumder et al., 2004).

This latter landmark study contributes several significant advances and forms the template for the future utilization of GEM models of premalignancies. First, highlighting the importance of studying "precancers," it clearly demonstrates the reversibility of a neoplastic process and prevention of invasive disease, even though the mPIN must first be recognized and characterized. Secondly, the reversibility was achieved by targeting the genetic abnormality driving the precancer phenotype. The AKT overexpression suggests that mTOR targeting was feasible. This approach constitutes a case study in rational, evidence-based, personalized therapy. Finally, the authors identified a biological readout of RAD001 activity by identifying a molecular signature of its action. They discovered that the hypoxia inducible factor (HIF)-regulated family of target genes, enriched in AKT transgenic mice, could serve as a predictive biomarker for monitoring therapy. Providing rigorous diagnostic criteria and validation of biological processes, GEMs may contribute to the design and implementation of both treatment and preventative protocols.

Similar studies have been performed in the mMIN systems of GEM. In these cases, the precancer has been identified, isolated and characterized with the test-by-transplantation (Medina et al., 2005; Namba et al., 2005). As might be anticipated from study of their tumors (Liu et al., 2005), erbB2 pathway mMIN are readily inhibited by Rapamycin (Namba et al., in press). The mammary gland is a hormone end organ and is particularly susceptible to SERMS, such as tamoxifin, and ovariectomy. Several groups have demonstrate that the precancers are equally sensitive to SERMSl (Medina et al., 2005; Namba et al., 2005). These types of study provide further proof of the principle that GEM can be used for chemoprevention.

Apc mutant mice have been produced by a mutagen, ethylnitrosourea and by genetic engineering (Taketo, 2006). The mice develop numerous precancerous lesions (variably termed polyps, adenomas, and aberrant crypt foci) (Alrawi et al., 2006) mostly in small intestine during the first few months of life. Many studies over the past 7 years have used the mutant mice for studying carcinogenesis, chemoprevention and therapy. They were first used for Cox-2 inhibitors and other human NSAIDs for chemoprevention studies NSAIDs which produced positive results (Jacoby et al., 2000) first in mice. Subsequently and presently human clinical trials have found similar results in polyposis patients (Steinbach et al., 2000; Phillips et al., 2002). Also many other studies report colon preclinical type studies for putative human colon chemoprevention agents using these mutant mice (Corpet and Pierre 2003, 2005).

DISCUSSION

The Precancer Workshop, consistent with previous discussions (Berman and Henson 2003a, 2003b) concluded that precancer has 5 defining properties:

1. Evidence exists that the precancer is associated with an increased risk of cancer.
2. A precancer has some chance of progressing to cancer, and the resulting cancer arises from cells within the precancer.

3. A precancer is different from the normal tissue from which it arises.
4. A precancer is different from the cancer into which it develops. A precancer may have some, but not all, of the molecular and phenotypic properties that characterize the cancer.
5. There must be a method by which the precancer can be diagnosed.

It should be noted that this definition of precancer includes the Group A, B and C lesions described by Foulds in his classical essays and monographs on neoplastic progression (Foulds, 1958, 1959). Thus, the wokshop definition of precancer includes categories of incipient, preneoplastic and malignant and is not limited to morphologically defined lesions.

Genetic engineering of mice has proven extremely useful for modeling human cancer. The technology has verified that mutant genes associated with human cancers also cause malignancies in other mammals (Cardiff et al., 2004). Genetic changes seen in human cancers when replicated in genetically engineered mice have resulted in new tumor phenotypes not previously observed in mice. The insertion or knockout of specific genes has yielded unique genotype-specific tumor phenotypes. Targeting of specific organs has led to the development of tumors in mouse organs, such as pancreas and prostate, that rarely develop spontaneous tumors. Examination of the tissues associated with these unique GEM tumors has revealed potential precancers in virtually every organ system and with every oncogene. The lesions have met the criteria of focality, atypia, and occurrence in association with tumor development as well as the temporal requirements of early appearance and progression with a morphological continuum terminating in malignancy. Consequently, the scientific community suddenly possesses a wealth of precancers available for study in a variety of organ systems. Each is initiated by one of a number of specific oncogenic molecules. Since most of the GEM have been constructed to test oncogenes or tumor suppressor genes known to be involved in human cancer, these precancers should become primary targets for understanding, treatment, and prevention and ideal representations of processes occurring in human precancers.

The "validity" of the GEM models has been discussed in some detail elsewhere (Cardiff, 2001; Green et al., 2002). The GEM mice are remarkable models of human cancers because they have been engineered with the same genes that are known to be associated with human cancers. Given that the investigator can traget and temporally control the "initiating" event, these models are gene- and pathway-specific molecular models in living animals. In an era filled with "molecular medicine" and anticipating "personalized medicine," GEM provides a very precise experimental test bed, beyond that which can be gained from spontaneous tumors and induced tumors in mice. The GEM models initiated by human-related oncogenes express the same RNAs and proteins found in the human counterpart and have morphologically similar tumors (Green and Hudson, 2005). On the other hand, GEM are mice and not humans.

The GEM models have reached such a stage of maturity that an increasing emphasis has been placed on using them for testing therapeutic and prevention strategies (Green and Hudson, 2005). Although numerous publications have reported various interventions in GEM, no systematic approach to such trials has been applied to these models. The primary focus of these studies has been treatment of the malignancy rather than prevention in the precancerous state. The potential use of these models was illustrated early by inhibition of colon cancer by Cox-2 inhibitors in the Apc model (Jacoby et al., 1996). In addition, the Apc model crossed to CEA Tg mice has been used to evaluate vaccine-based immunotherapy that reduced the number of tumors (Greiner et al., 2002). Recent studies using mTOR inhibitors indicate inhibition of precancerous growth is possible (Namba et al., 2006). Studies using mouse models of mPIN, discussed here, have demonstrated a rational, evidence-based strategy for chemoprevention.

Despite their usefulness, the GEM models, characterized by prolonged latencies and multiple tumors, have proven relatively expensive and cumbersome for preclinical trials. Models with tumors exhibit multiple precancers, each of which may exert a unique biological potential that must be understood. Although many investigators have recognized precancers in their model systems, few have systematically studied the biology of neoplastic progression. When elucidation of this sequential development occurs, the models will become more amenable to therapeutic intervention.

The transplantation systems not only have contributed to the establishment of several therapies for the treatment of human cancers, but they also have proven to be ideal for investigations leading to the treatment and prevention of precancer. Transplantation permits the in vivo expansion of molecularly induced, biologically defined, clonal populations of precancerous tissues. In vivo imaging of the transplant can be performed relatively easily with little background noise (Abbey et al., 2004). Longitudinal studies in individual animals increase the statistical power without requiring large animal cohorts. Since the lesions are transplanted into syngeneic hosts, their sera become a primary source for discovery of potential surrogate biomarkers that can be validated using the native transgenic mouse. The transplant systems can provide the resources needed for development of customized, molecular-based, intervention strategies as well as sufficient numbers of test subjects for relatively rapid, high-volume preclinical trials on defined tissues. Since these outgrowths are also associated with emerging malignancies, the entire spectrum of neoplastic progression becomes available for scrutiny. Different strategies for intervention and prevention can be developed and modified in the mouse before clinical trials.

In summary, mice develop well-characterized precancers that are proving useful for extrapolation to humans. With genetic engineering, investigators have identified potential precancers in virtually all mouse organs targeted. Since these genes are known to be associated with human cancers, the mouse affords the research community a marvelous opportunity to understand the biology of precancers in a controlled, homogeneous, biologically intact, whole animal. Since the precancerous lesions can be identified and expanded in a syngeneic system, the GEM, providing molecular and biological proof-of-principle will continue to afford the most valid biological test system for molecular medicine.

Biological interpretations of precancerous lesions in the mouse are frequently quite distinct from those applied to human pathology. For example, the terms hyperplasia and metaplasia are not included in the human classifications of

lung cancer; in mouse pulmonary pathology, however, these lesions are interpreted as an important potential constituent of carcinogenesis and are included in some classifications, as previously reviewed (Nikitin et al., 2004). While such discrepancies may be explained to some extent by differences between human and mouse biology; they are derived chiefly from differences in interpretation of precancerous lesions by human, comparative, and experimental pathologists. Closer interactions among pathologists and investigators, combined with advanced cross-species phenotyping of lesions using transcriptome and proteome, should allow comparative assessments of precancer in mice and humans and lead ultimately to transferral of knowledge gained in mouse models to clinical settings.

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