The Value of Outbred Rodent Models in Cancer Research

Chatzistamou I[1], Farmaki E[2], Kaza V[3] & Kiaris H[2,3]*

[1]Department of Pathology, Microbiology and Immunology, School of Medicine, University of South Carolina, SC, USA.

[2]Peromyscus Genetic Stock Center, University of South Carolina, SC, USA

[3]Department of Drug Discovery and Biomedical Sciences, College of Pharmacy, University of South Carolina, SC, USA.

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***Correspondence**: Hippokratis Kiaris PhD, CLS 713, 715 SUMTER STREET, COLUMBIA SC 29208-3402 Phone: 803 3611 781 Email: kiarish@cop.sc.edu

Abstract

Mouse models of breast cancer are valuable research tools but their usefulness is restricted by a series of features inherent to their physiology, such as low endogenous estrogens and genetics (inbred status). Depending on the specific questions asked outbred rodents like *Peromyscus* may provide answers that laboratory mice cannot.

Conventional inbred and outbred strains of laboratory mice represent the golden standard models in preclinical cancer research. More than 95% of studies are performed in mice (genus *Mus)*, followed by a small portion of studies performed in rats (genus *Rattus*) (1). The studies usually involve induction of tumor growth following genetic manipulation, chemical mutagenesis or viral infection of experimental animals that eventually develop primary cancers of mouse origin. Alternatively, these studies involve inoculation of cancer cells, preferably of human origin, to study human cancers in the context of the whole organism, a preferred approach to screen and assess the anticancer activity of experimental drugs (1).

To improve modeling of human cancers *in vivo*, investigators continuously attempt to enhance mouse models by focusing typically either on the site or on the type of the inoculum. Therefore, orthotopic growth of cancer cells is used to study primary cancer within its natural microenvironment, and cancer cell inoculation at secondary sites is used to study metastasis. Inoculation of cancer cells admixed with stroma is also common, and more recently, the growth of patient-derived xenografts being developed offers unique opportunities to study naturally occurring human cancers in animals (2). Nevertheless, the hosts, usually immunocompromised to various degrees, and always remain genetically identical, which constitutes a non-human bias

that is common to all experimental studies (3). Depending on the cancer being studied, additional manipulations might be required, such as supplementation of the host with estrogens to facilitate the growth of hormone-sensitive breast cancers, a strategy that has, however, several limitations such as the estradiol-associated toxicity and the maintenance of steady and not- fluctuating estradiol levels (4,5).

Despite their undoubted value, these approaches suffer from major deficits that drastically limit the collection of meaningful information that can be readily applicable to people. For example, the impact of the host in tumorigenesis is very likely biased. Laboratory mice are inbred or of limited genetic diversity (3). This is advantageous in terms of the uniformity of the results obtained and the extraction of meaningful mechanistic conclusions, as limited and specific variables are assessed during the course of the experiments. However, the heterogeneity in the host's genetic make-up, as seen in human populations, cannot be factored in and accounted for in such studies. Growth of human tumors in mice only models a very specific and deviant form of the disease that occurs in diverse populations. With the exception of studies addressing the contribution of specific germline mutations in cancer growth, the impact of the host in tumorigenesis cannot be appreciated and remains largely unexplored.

A solution to these limitations may be the use of genetically diverse populations as hosts for tumor growth. Animals of the genus *Peromyscus* are maintained as outbred stocks in captivity (6). Pharmacological immunosuppression by cyclosporine A (CsA), following a strategy that in the past was used successfully to immunosuppress both mice and rats (7,8), facilitates the growth of human breast tumors in *Peromyscus californicus* (Figure 1) (9). The resulting tumors are rich in stroma and considerably more heterogenous histologically than those in mice, underscoring the impact of the host in specific tumor histopathological profiles.

Estrogen receptor negative and estrogen receptor positive breast cancers grow without exogenous estrogen supplementationin a *P. californicus* model with moderately higher circulating estradiol levels than mice but lower than those of pre-menopausal women (9,10) . In this model, estrogen sensitivity had been retained as evidenced by the efficacy of the Fulvestrant (antiestrogen) and Letrozole (aromatase inhibitor). This implies that even moderate elevation in the levels of endogenous estradiol generates permissive conditions for cancer growth within an appropriate microenvironment. This is particularly relevant to human breast cancer in which about half of the cases occur in postmenopausal women that have a range of endogenous estradiol (E2) levels similar to *Peromyscus*. Removing exogenous estrogen supplementation in mice, not only offers a more physiological setting for tumor growth, but also removes common toxicity problems of estradiol (4,5). It is noted that the requirement for exogenous E2 supplementation, as shown recently, can be bypassed in conventional mice by the intraductal administration of cancer cells in a model that can sustain the growth of hormone sensitive breast cancers without exogenous estrogen administration (11,12). However in this model the genetic homogeneity of the hosts remains as a prerequisite as heavily immunocompromised- and therefore inbred - animals have to be used. Some of these limitations may be addressed by using other outbred mice models, such as the CD-1 mice. Despite their genetic diversity, most outbred mouse strains, including CD-1 mice, can be trace back to 9 breeders, 2 males and 7 females, which may also limit genomic variation (13).

The power of using an outbred model is also exemplified in the efficacy of conventional therapies. Comparison of tumor growth in animals treated with Fulvestrant or Letrozole with that of untreated controls showed that in the former, despite the efficacy of the therapy, variance in

tumor growth was elevated as compared to the untreated controls (9). The acquisition of drug resistance and the clonal expansion of cancer cells occasionally contributes to increased variation in tumor growth rates during therapy. Since the outbred Peromyscus study (9) lasted only for 3 weeks clonal selection likely does not account for the variance but is likely attributed to the host's differential impacts, such as genetic heterogeneity, microenvironment and to its conceivable role as modulator of drug efficacy.

Administration of CsA and an immunocompromised state of the animal may undoubtedly interfere either directly or indirectly with the growth of cancer cells, and if in combination with cytotoxic drugs, may mask the drug effects. Indeed, breast tumor growth in animals treated with CsA was reduced as compared to immunosuppressed animals (9). Whether this difference in growth rates was due to the cytotoxic effects of CsA in the cancer cells or due to the partial immunosuppression of *Peromyscus* by the CsA and evidenced by the lymphocytic infiltrations in the tumors, remains to be established. However, depending on the exact question asked this may not be of primal concern. Exploiting outbred animal models can facilitate studies that illuminate aspects of tumorigenesis that have been ignored or only indirectly addressed. For example, fundamental questions related to whether the pressure inflicted by systemic or paracrine mechanisms during tumor growth or during therapy results preferentially in the adaptation of the cancer cells or in their clonal selection, can readily be addressed by comparing the variation of the tumors' histological and molecular profile after growth in genetically diverse animals. To that end, re-growing tumors that had previously grown in *Peromyscus* in conventional immunocompromised mice and comparing their profiles will allow discrimination of whether molecular and histological changes were adaptive or proceeded through selection (Figure 2). Incorporation of different therapies in such studies would also allow evaluating if the response of

specific cancers to different drugs favors primarily adaptation or selection. Such information may have imminent implications in the clinic: By knowing if given drugs favor selection or adaptation, oncologists might better predict the most appropriate sequence for drug administration in complex therapeutic schemes. Finally, the genetic diversity of the animals in combination with specific features in the resulting tumors may allow genome association studies to reveal modifiers of tumor growth originating from the host.

In summary, genetically diverse outbred species such as *Peromyscus* may be particularly useful in cancer research by providing information important to patient strata that cannot be obtained by using conventional genetically homogenous mice as models.

Figure legends

Figure 1. *Peromyscus californicus*. This is one of the largest, species of *Peromyscus* weighing about 45g. It lives more than 4-5 years in captivity, it is monogamous and provides paternal care of offspring. It is commonly used for behavioral studies.

Figure 2. Outbred hosts and the assessment of selective or adaptive changes in tumorigenesis. Tumors derived from human breast cancer cells injected in genetically diverse *P. californicus* acquire a profile influenced by the individual hosts. Subsequently explants are introduced into inbred immunocompromised mice. The tumors in mice can either retain original molecular characteristics recorded while growing in *P. californicus* indicating selection, or they will abolish these differences and acquire a more uniform profile indicating adaptation.

Glossary

Inbred Mouse Strain: Strains of mice at which individual animals are genetically identical due to prolonged inbreeding. Experimentally this is usually attained by extensive, more than 20, consecutive mattings between brother-sister or parent-offspring matting and results in similarity that exceeds 99%.

Outbred Mouse Strain: These strains are characterized by some degree of genetic diversity which is desirably high. The genetic diversity of outbred stocks is usually maintained by utilizing rotational breeding schemes that minimize inbreeding.

Orthotopic Models: Tumor models at which the cancer cells are implanted at the same anatomical location from which the primary tumor has been derived.

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