Mouse Models of Cancer

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Abstract

Knowledge about the nature of cancer has been increasing, in part because of the development and use of mouse models of cancer that recapitulate the situation of cancer patients in the laboratory and help to elucidate the molecular mechanisms underlying carcinogenesis. Mouse models have been, and will be, used in cancer research until we find a cure for cancer. Over the last two decades in particular, mouse models of cancer have played an increasingly significant role in research aimed at translating achievements in basic science to clinical practice to establish the best therapies and assessments to overcome cancer. One of the most powerful approaches in this field is the use of genetically engineered mouse models carrying the genetic alterations identified in clinical specimens, which has become generalized and accelerates the development of novel therapies and diagnostic methods.

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In this section, we first provide an overview of the mouse models of cancer and then shed new light on the genetically engineered mouse models that have been used in pancreatic cancer research to obtain clues regarding a cure and to allow the detection of this disease at an early stage.

Keywords
Mouse models of cancer • Genetically engineered mouse models • Pancreatic cancer • Pancreatic duct adenocarcinoma • KRAS-driven PDAC models • Temperature-sensitive TAg

Introduction

Mouse models of cancer have been largely classified into two categories: (1) mice that carry implanted cancer cells and (2) mice that develop cancer. The mouse models used currently in cancer research are summarized in Table 1. For the preparation of the former, both athymic mice (i.e., nude mice) and severe combined immunodeficiency mice (i.e., SCID mice) have been used, because human cancer cell lines are usually applied to these mice (which are used as recipients) via xenotransplantation experiments. Conversely, the latter develop cancer usually via either exposure to extrinsic stresses, such as chemical carcinogens, infection with microorganisms, and radiation, or intrinsic stresses, such as transgenic expression of oncogenic molecule(s) and disruption of tumor suppressor molecule(s). Currently, genetically engineered mice harboring genetic alterations identified in clinical specimens are often used in cancer research as genetically engineered mouse models (GEMMs) . Accordingly, cancer research performed using GEMMs has succeeded not only in elucidating the mechanisms of carcinogenesis but also in illustrating the biological significance of senescence and chronic inflammation as cancer-promoting factors. As this tendency is particularly evident in pancreatic cancer research, we will describe mouse models in the context of this disease.

Table 1 Mouse cancer models

1. Mice that develop cancer
(i) Extrinsic stresses to include cancer
(a) Infection of microorganism (virus, bacteria, protozoan)
(b) Administration of chemical reagents
(c) Radiation, UV
(ii) Intrinsic stress to include cancer
(a) Transgenic expression of oncogenic molecules(s)
(b) Disruption of tumor suppressor molecule(s)
2. Mice carry implanted cancer cells
(i) Orthotropic and heterotopic
(ii) Xenotransplantation or not

Principles of Mouse Models in Pancreatic Cancer

Pancreatic duct adenocarcinoma (PDAC) accounts for more than 90 % of cancers of the pancreas and exhibits ductal phenotypes, such as the formation of glandular structures and the production of mucin. Despite the conspicuous progress in medical procedures that generally improve the prognosis of cancer patients, the prognosis of patients with PDAC has not improved over the past 30 years. Moreover, the aggressive clinical course of PDAC remains uncontrolled, and this type of cancer exhibits a catastrophic progression, with a 5-year survival rate that remains below 5–7 % (Real 2003; Siegel et al. 2011; Vincent et al. 2011).

Over the last two decades, studies that used clinical specimens of PDAC unveiled molecular mechanisms that underlie its carcinogenesis. A series of analyses performed using molecular techniques have identified clinically relevant mutations, such as those in the Kirsten rat sarcoma viral oncogene homologue (*KRAS*), cyclin-dependent kinase inhibitor 2A (*CDKN2A*), *p53*, and SMAD family member 4 (*SMAD4*)/*DPC4* genes, which illustrated the multistep process of PDAC carcinogenesis (Hezel et al. 2006). It is noteworthy that PDAC results from abnormalities in both the KRAS/Rb/E2F pathway and the transformed 3T3 cell double minute 2 (MDM2)/p53 pathway. In addition, it is important to note that the genetic alterations mentioned above were detected not only in PDAC but also in pancreatic intraepithelial neoplasia (PanIN), which is considered to be a potential precancerous lesion of PDAC.

Regarding GEMMs that develop PDAC, a series of studies using mice that are termed "KRAS-driven PDAC models" have illustrated the multistep process of pancreatic carcinogenesis. In fact, the multistep process that leads to the development of PDAC is accelerated by the ablation of the *CDKN2A*, *TP53*, and *SMAD4*/*DPC4* genes in KRAS-driven PDAC models (Morris et al. 2010; Vincent et al. 2011). Moreover, these models have been used to show that the production of hyaluronic acid may be involved in drug resistance in PDAC[6]. Furthermore, the "cell of origin for PDAC" was identified by tracing a green fluorescence protein (GFP) that was induced in specific cell lineages in KRAS-driven PDAC models (Gidekel Friedlander et al. 2009; Reichert and Rustgi 2011). Thus, KRAS-driven PDAC models have contributed to an increase in knowledge about pancreatic cancer. However, as the KRAS-driven PDAC models are merely a mouse model that illustrates a multistep process of carcinogenesis that requires more than 6 months, these models have been used to elucidate the molecular mechanism of the cancer-suppressive roles of senescence and the cancer-promoting effects of chronic inflammation during precancerous stages (Guerra et al. 2011).

A New Mouse Model of Pancreatic Cancer

Taking into account the basic idea described above, a new model that recapitulates the catastrophic clinical course of human PDAC patients was required to change the current situation of pancreatic cancer; in this context, we have successfully

generated a new mouse model that develops PDAC with a catastrophic progression (Introduced on AACR Annual Meeting 2011). ThisGEMM harbors *loxP-βgeo-stop-loxP-tsTAg*(*LBSL-tsTAg*) (Yamaguchi et al. 2008), *loxP-STOP-loxP-Kras*^{G12D} (*LSL-Kras*^{G12D}) (Jackson et al. 2001), and pancreatic and duodenal homeobox 1 (*Pdx1*)-*Cre* (*TKC* mice). Via *Pdx1*-promoter-mediated Cre/*loxP* recombination, this GEMM expresses both the temperature-sensitive TAg (thermosensitive TAg (tsTAg)) from SV40 virus (Jat and Sharp 1989) and Kras^{G12D} specifically in epithelial cells of the pancreas. *TKC* mice develop rapid progressive pancreatic ductal adenocarcinoma and die within 1 month, whereas mice that express tsTAg alone (*TC* mice) develop a normal pancreas without neoplasms or a ductal cell phenotype.

A transgenic mouse that expresses tsTAg under the control of the *H-2k*^{b} promoter was initially established to prepare immortalized cell lines to assess the biological functions of normal cells in in vitro experiments (Jat et al. 1991), because tsTAg inhibits the functions of the Rb family of molecules (Rb1, p107/Rbl1, and p130/Rbl2), i.e., the release and activation of the E2F transcription factor, and abolishes the function of p53 via direct binding. Similar to lymphatic endothelial cells (Yamaguchi et al. 2008), we prepared immortalized pancreatic duct cells from *TC* mice that expressed both cytokeratin (CK) 19 and CK8. It is noteworthy that the duct cell lines formed spheres and ductal structures that were similar to either intra- or interlobular ducts, rather than to structures of the main pancreatic ducts, in three-dimensional culture using collagen (Fig. 1), whereas cancer cell lines from *TKC* mice yielded ductal structures with irregular and disarrayed branches (similar to those of primary tumor tissues in *TKC* mice). Thus, the *TKC* mouse is a new model that exhibits progression of PDAC via a de novo carcinogenesis pathway.

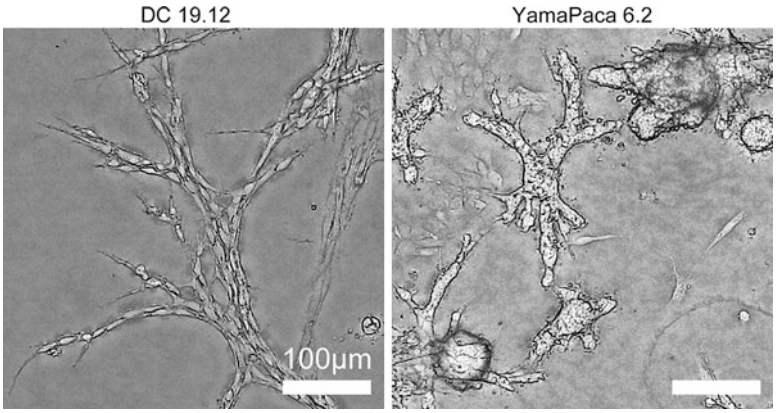

Fig. 1 DC 19.12 and YamaPaCa 6.2 cells formed ductal structures in three-dimensional (3D) culture using collagen gel. DC 19.12 is an immortalized pancreatic ductal cell line, whereas YamaPaCa 6.2 is a cell line from PDAC that developed in the mouse pancreas. In a 3D gel culture, DC 19.12 cells formed ductal structures that were similar to the intralobular pancreatic ducts, whereas YamaPaCa 6.2 cells formed tubular structures with irregular and disarrayed branches. The term "YamaPaCa" stems from "Yamaguchi-established pancreatic carcinoma"

Because our newly established mouse model showing de novo carcinogenesis and an appropriate pair of cancer cell lines and immortalized cells of the cell of origin of PDAC facilitated the distinction of cancer-specific changes, the development of new glycobiomarkers and molecular targets to overcome this catastrophic disease will be promoted by these models.

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