Meeting Report

Cancer Research

Mouse Models of Human Cancer

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Abstract

The Helmholtz Alliance Preclinical Comprehensive Cancer Center (PCCC; www.helmholtz-pccc.de) hosted the "1st International Kloster Seeon Meeting on Mouse Models of Human Cancer" in the Seeon monastery (Germany) from March 8 to 11, 2014. The meeting focused on the development and application of novel mouse models in tumor research and high-throughput technologies to overcome one of the most critical bottlenecks in translational bench-to-bedside tumor biology research. Moreover, the participants discussed basic molecular mechanisms underlying tumor initiation, progression, metastasis, and therapy resistance, which are the prerequisite for the development of novel treatment strategies and clinical applications in cancer therapy. *Cancer Res; 74(17); 4671–5. ©2014 AACR.*

Introduction

The availability of advanced preclinical tumor models has emerged as one of the most rate-limiting steps in the translation of basic tumor biology discoveries into diagnostic and clinical applications. Therefore, the gap between basic and clinical research needs to be bridged to cross what has been called "the valley of death" (1), which separates basic researchers and clinicians. This ambitious goal can only be achieved by bringing basic researchers and clinicians together to overcome the translational gap. The Helmholtz Alliance Preclinical Comprehensive Cancer Center (PCCC; www.helmholtz-pccc.de) has, in 2013, implemented a nationwide consortium, in which basic and clinical researchers work closely together to establish and validate novel preclinical cancer models. The units of the consortium thereby aim at developing superior mouse models, which truly mimic the natural course of human tumor initiation, growth, and metastatic dissemination. Focusing on the key issues of contemporary basic and clinical oncology research, such concerted effort holds great promise to substantially advance translational oncology research. In this spirit, the "1st International Kloster Seeon Meeting on Mouse Models of Human Cancer" was held in the Seeon monastery (Germany) in March 2014. A total of 100 senior and junior scientists in the field, selected on the basis of applications and invitations, transformed the tranquil monastery for 4 days into a tumor biology think tank with high-profile plenary lectures and plenty of room for informal scientific discussions in the bar and in the bowling alley. The meeting combined the discussion of novel tumor mouse models and the prerequisite for their development and implementation, the understanding of the basic principles and the molecular mechanisms of tumor initiation, progression, and metastasis. Nineteen keynote speakers highlighted the most urgent topics in the field, and numerous presentations by young investigators selected on the basis of their submitted abstracts complemented the program (see Supplementary Data). It would be beyond the scope of this short meeting report to summarize all of the many exciting presentations of the meeting. Moreover, a lot of unpublished data were presented that would be inappropriate to spell out here in detail. Instead, the following report briefly summarizes some of the presentations that coherently point toward major overall development in the field—related to (i) novel advanced mouse models of human cancer, (ii) tumor cell heterogeneity, (iii) the role of inflammation toward tumorigenesis and tumor progression, and (iv) synthetic lethality and other novel technologic advances.

Next-Generation Preclinical Mouse Models of Cancer

Preclinical drug development is limited by the restricted availability of suitable animal tumor models that adequately mimic the evolution, the growth kinetics, the molecular and functional heterogeneity, and the overall functional and phenotypic properties of human tumors, including their response to drug candidates. Available and emerging mouse models of cancer can be hierarchically clustered into different stages of development and sophistication (Fig. 1). First-generation mouse models use xenotransplants or syngeneic transplants, in which tumor cells are engrafted subcutaneously or orthotopically. The second generation of genetic mouse models [genetically engineered mouse models (GEMM)] allows for

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Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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doi: 10.1158/0008-5472.CAN-14-1424

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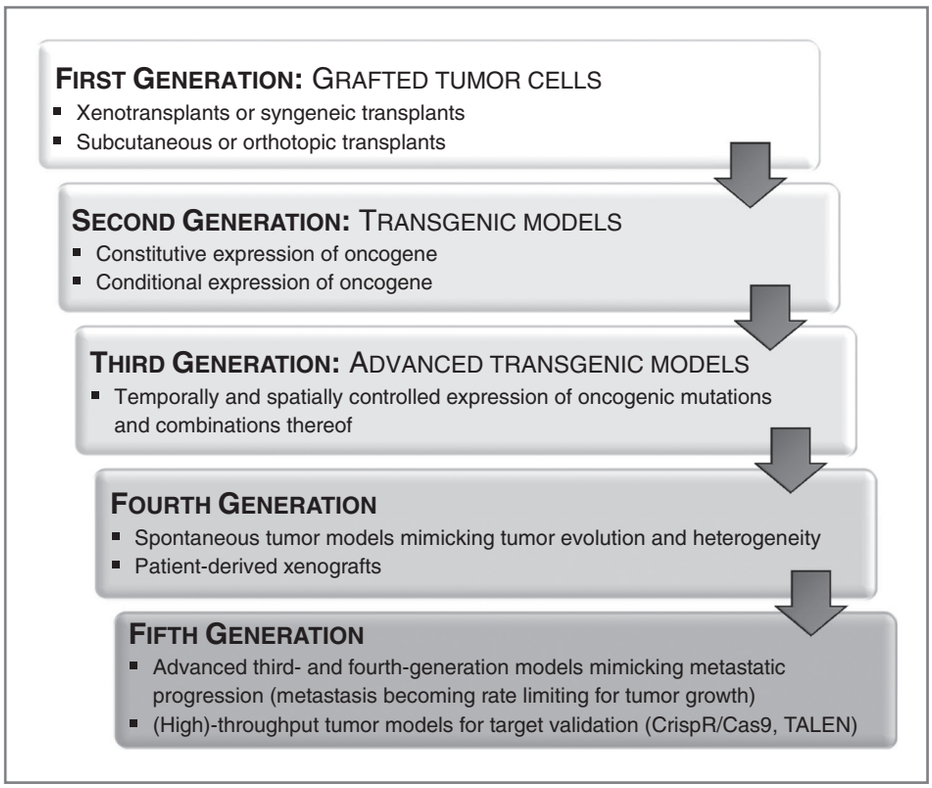

Figure 1. Development and advancement of mouse models of human cancer (see the text for details).

the constitutive or conditional expression of oncogenes or shut down of tumor suppressors. The third generation offers the possibility of a temporally and spatially controlled expression of oncogenic mutations, including the combinatorial expression of different mutations. The fourth step marks the development of spontaneous tumor and patient-derived xenografts (PDX) models. Next will be the development of fifth generation of mouse tumor models, which not only mimic the natural cause of tumor evolution and heterogeneity, but also the natural progression toward metastatic dissemination, dormancy, and growth (preferably in a way that metastasis becomes rate limiting for the course of the experiment).

Great effort is being made at implementing *in vivo* screening techniques with higher throughput capacity for target validation purposes. Mariano Barbacid (CNIO, Madrid, Spain) demonstrated that genetic-based strategies have significant advantages over classic pharmacologic approaches because the observed effects are always mechanism based and avoid off-target effects. Moreover, genetic models do not rely on the quality of drugs or inhibitors. Thus, mechanism-based target validation studies are an essential prerequisite for the development of targeted therapies. Advanced mouse models developed by the Barbacid laboratory have enabled the demonstration that K-Ras signaling in pancreatic adenocarcinoma and lung adenocarcinoma proceeds through C-Raf and not equally through all subtypes of Raf, as proposed earlier (2). A-Raf and B-Raf are dispensable for this process, making C-Raf a suitable therapeutic target for targeting K-Ras signaling. Investigation of epidermal growth factor receptor (EGFR), an upstream effector of Ras signaling, showed that EGFR targeting has no therapeutic value in K-Ras–driven lung adenocarcinoma and intestinal tumors, but that it is essential for the initiation of pancreatic tumors. This coincides with clinical observations. It also demonstrates that every tumor entity underlies its own mode of action. Mouse models of human cancer are, thus, perfectly suited to dissect these pathways and their role during tumorigenesis in different tumor entities.

Dissecting the multistep pathways of tumorigenesis is important not only for the understanding of the causal pathogenesis of tumors but also to decipher the development of therapy resistance. Inherited breast and ovarian cancer are associated with the loss of *BRCA1* or *BRCA2*. Jos Jonkers (NKI, Amsterdam, the Netherlands) presented GEMM and PDX models for *BRCA1*–deficient triple-negative breast cancer (3). These mice develop breast tumors, which are characterized by genetic instability and hypersensitivity to DNA-damaging agents, as similarly observed in humans. In human patients with tumor, platinum drugs and PARP inhibitors are not curative. The tumors eventually relapses and acquires drug resistance. Using the newly developed mouse models, DNA methylation analysis and whole-genome sequencing approaches demonstrated that the resistance to the PARP inhibitor olaparib is induced by different mechanisms, including the upregulation of drug efflux transporters, genetic reversion of truncating mutations in *BRCA1*, rescue of DNA end resection by loss of *53BP1*, and hypermorphic *BRCA1* activity. These results clearly demonstrate that GEMM and PDX models will improve accurate patient stratification and help to develop treatment strategies that counteract or prevent resistance to PARP inhibitors.

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David Tuveson (Cold Spring Harbor Laboratory, New York, NY) emphasized the importance of novel mouse models for the treatment of pancreatic cancer. These preclinical models will help to uncover novel aspects of tumorigenesis. Pancreatic stellate cells (PSC) play a key role in the development of pancreatic adenocarcinoma. PSCs are responsible for the collagen- and glycosaminoglycan-rich extracellular matrix, which results in vessel compression, eventually leading to hypovascularization, a hallmark of pancreatic cancer and a rate-limiting factor for drug delivery. Therefore, targeting the extracellular matrix and reducing its stiffness may be a promising approach to increase the efficacy of therapy. He showed that FG-3019 promotes cleavage of connective tissue growth factor, resulting in increased survival in combination with gemcitabine, supporting the idea that targeting extracellular matrix components may be a promising future therapeutic intervention (4). The critical role of microenvironmental determinants for tumor growth was also emphasized by Andreas Trumpp (DKFZ, Heidelberg, Germany), who showed that the exploitation of PDX models marks an important approach toward the advancement of personalized oncology. The interplay between hematopoietic cells and their myeloid stem cells (MSC) drives myelodysplastic syndromes (MDS). Using PDX models, he could show that patient-derived hematopoietic cells instruct healthy MSCs. These MSCs acquire MDS-like features and in turn promote tumor progression (5). Therefore, interfering with microenvironmental shaping and inhibiting the interaction with the MSC niche may offer great therapeutic intervention strategies.

Allan Bradley (Sanger Institute, Cambridge, United Kingdom) and Gavin Thurston (Regeneron Pharmaceuticals, Tarrytown, NY) presented humanized mouse tumor models, in which the loci for the immunoglobulin heavy and light chain were substituted by the corresponding human genes (6). These mice provide not only a platform for therapeutic antibody discovery, but they are also a valuable tool to test immunomodulatory therapeutics. A major step toward bench-to-bedside clinical translation was also presented by Andrea Lunardi (Harvard Medical School, Boston, MA), who introduced the "co-clinical trial project" (7). This initiative comprises studies that are performed in several genetic mouse models conducted in parallel with human patients enrolled in ongoing phase I and II clinical trials. This platform improves the velocity and the outcome of personalized treatments and helps to identify genetic and molecular determinants mediating drug resistance. This approach will also significantly contribute to overcoming the current obstacle of therapy resistance and enable oncologists to tailor novel, optimized combinatorial therapies based on patient stratification.

Tumor Heterogeneity and the Nature of the Tumor-Initiating Cell

It is increasingly recognized that tumor heterogeneity plays an important role in tumor progression and treatment resistance. Tumor heterogeneity marks the fact that the different tumor cells within the tumor may genetically, phenotypically, and functionally be vastly different, and that continuous tumor evolution contributes to tumor heterogeneity and selection of clones with growth advantages (e.g., therapy resistance). Such evolutionary processes contribute to malignancy and make tumors an oftentimes treatment-escaping deadly disease. Tumor heterogeneity may also originate from treatment-resistant cancer stem cells (CSC). Incorporating the understanding and knowledge of tumor heterogeneity will, thus, help to guide the development of more refined treatment strategies to yield better therapeutic outcome.

Lung cancer may grow as non–small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). Although SCLC compromises only 20% of the cases, it is among the most lethal malignancies. This is due to the fact that it displays an early high metastatic potential. Moreover, it exerts chemotherapy resistance after relapse. Anton Berns (NKI, Amsterdam, the Netherlands) and colleagues have developed a mouse model for SCLC by inducible loss of *p53* and *Rb1*. This model shares a number of features with the human disease, including distinct morphologic characteristics, marker expression profile and metastatic spread to specific organs. Using this model, they asked to which extent the tumor heterogeneity affected the overall tumor population and its properties. Toward this end, loss of *p53* and *Rb1* was targeted to Clara, neuroendocrine, and alveolar subtype II cells. Their work demonstrated that predominately neuroendocrine cells served as the cell of origin for SCLC. In contrast, activated K-Ras drives alveolar and Clara cells to serve as cell of origin for adenocarcinoma, an NSCLC subtype, demonstrating that cell type–specific features and the oncogenic lesion determine the tumor-initiating capacity of progenitor cells and that the cell of origin determines the malignant properties of a tumor (8).

Anton Berns' presentation in support of the clonal evolution model was complemented by Haikun Liu's lecture, whose laboratory (DKFZ, Heidelberg, Germany) studies in novel mouse models the development of high-grade glioma originating from CSCs. He introduced a mouse model of high-grade glioma that allows stem cell–specific targeting through the nuclear receptor tailless (Tlx), which is a neural stem cell–specific transcription factor (9). Interestingly, *tlx*-positive cells in primary brain tumors are mostly quiescent, but have the capacity to self-renew and give rise to *tlx*-negative tumor cells, suggesting that they are brain tumor stem cells (BTSC). BTSC-specific loss of tlx in mice leads to the loss of self-renewal capacity and to the activation of signaling pathways, which induce cell-cycle arrest and neural differentiation or cell death. Moreover, the knockout mice showed a prolonged survival. Tlx also serves as a new prognostic marker for a reduced survival in high-grade glioma and is a valuable therapeutic target for the treatment of glioblastoma.

Luis Parada (University of Texas Southwestern, Dallas, TX) also emphasized that tumor heterogeneity opens the door for new therapies. Conventional cancer therapy hardly qualifies as specific anticancer therapy, but merely acts as an antimitotic therapy. On the premises that glioblastoma arises from CSCs, these therapies are inefficient because they do not target nondividing cells such as quiescent CSCs. Thus, to develop an efficient therapy for glioblastoma, one needs to understand which alterations occur in the cell of origin before tumor

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initiation and how to single out these cells. The cell of origin will determine the growth rate of the tumor, depending on whether it originates from CSCs (fast growth) or from oligodendrocyte progenitor cells (slower growth). Therefore, understanding the origin of a tumor and its specific properties will advance tumor therapy by specifically targeting the tumor-initiating cell type (10).

Inflammation: Friend or Foe?

At first glance, inflammation may be considered a beneficial and protective response of an organism toward pathogens. Furthermore, it is also associated with regenerative processes, for example, in the context of wound healing. In analogy, tumors have been considered as "wounds that won't heal" (11), and inflammation is one of the hallmarks of cancer (12). Yinon Ben-Neriah (Hebrew University, Jerusalem, Israel) introduced the concept of senescence-inflammatory response. He showed that this form of para-inflammation drives tumorigenesis in a mouse model of intestinal cancer and is comparable with observations made in human intestinal cancer (13). Interestingly, the switch between tumor-promoting versus tumor-inhibiting effects of para-inflammation depends on the action of p53. Upon loss of *p53*, para-inflammation drives tumorigenesis. Interestingly, this novel concept of senescence-inflammatory response has been included in the *Cancer Cell—Best of 2013* issue.

Raghu Kalluri's (MD Anderson Cancer Center, Houston, TX) presentation shed new and unexpected insights into the role of chronic inflammation on tumor progression. Tissue damage causes a wound-healing response. However, chronic tissue damage may lead to tumor growth. Myofibroblasts, collagen I, and other determinants of the extracellular matrix are necessary for wound healing. Experiments in a mouse model of genetically engineered myofibroblast ablation resulted in much more dedifferentiated tumors and, consequently, the survival of mice was reduced compared with wild-type mice. This is also observed in patients suffering from pancreatic ductal adenocarcinoma, in whom a low number of myofibroblasts correlates with poor prognosis. Therefore, the process of wound healing and its associated components, myofibroblasts and collagen I, can be considered as negative regulators of tumor progression (14).

Synthetic Lethality: A Key to New Therapy Approaches

Chemotherapy is mostly not curative, because it fails to eliminate all tumor cells. Some tumor cells escape therapy by undergoing cellular senescence. These cells may be considered a "time bomb." First of all, senescent cells secrete high levels of inflammatory proteins. Second, they can be reactivated and cause tumor relapse. Clemens Schmitt (Charité & MDC, Berlin, Germany) reported that senescent lymphoma cells have an extremely high energy demand and heavily rely on glucose (15). Moreover, they require an intact autophagy machinery to digest the excessive amount of toxic proteins they produce. By blocking either glucose utilization or autophagy, tumor cells are selectively killed by apoptosis. The high metabolic demands are unique for tumor cells and, therefore, glucose deprivation or autophagy inhibition does not affect normal body cells. Synthetic lethal therapy approaches will have a great benefit for patients with cancer not only by its increased efficacy but also by its reduced side effects. Synthetic lethal approaches are certainly at the cutting edge between preclinical and clinical research.

Emerging Technologic Advances

Despite the mechanistic power of GEMMs, a critical limitation is their limited throughput capacity and the oftentimes quite time-consuming procedures. Depending on the complexity of the genetic alterations, the establishment and validation of a novel GEMM may take years. Therefore, fast and reliable animal models are urgently needed to test genes for their oncogenic potential in a higher throughput manner than is presently doable. The CRISPR/Cas9 system allows the faster functional testing of candidate genes obtained from whole-genome sequencing. Similarly, large-scale screening approaches such as transposon-based insertional mutagenesis applying sleeping beauty and/or PiggyBac technology (presentation by Roland Rad, Technical University, Munich, Germany; ref. 16) or RNAi screens (presentation by Lars Zender, University of Tübingen, Tübingen, Germany; ref. 17) have advanced the identification of genetic networks of tumorigenesis. This offers the possibility to study tumor initiation, progression, and metastasis and treatment resistance in larger genome-wide screens.

Bottlenecks and Perspectives

Taken together, the meeting clearly showed that advanced preclinical mouse models have a central position in linking basic discovery research on the molecular mechanisms of tumor initiation, progression, and therapy resistance with the translational development of novel therapeutic and diagnostic procedures and strategies. In turn, the meeting also revealed some limitations of contemporary mouse tumor models, which heavily focus on the early-stage disease and do not address the issue of clinical metastasis, which is the major cause of human cancer–related morbidity. As suggested in Fig. 1, the development of what may be considered fifth-generation advanced mouse tumor models, mimicking metastatic progression so that metastatic progression and not the primary tumor becomes rate-limiting for tumor growth, is in tremendous need. Better preclinical metastasis models will not just advance translational tumor biology research, but hold great promise to fundamentally advance basic metastasis research. On a different note, the dramatic progress to map the landscape of mutations in human tumors as pursued in the International Cancer Genome Consortium (ICGC) is awaiting a corresponding systematic approach in mouse tumor models. Systematic mouse/human genomic comparison may not just contribute to better discriminate between driver and passenger mutations, but will also be important for the overall validation of preclinical mouse tumor models, particularly in implementing novel biologic therapies and immunotherapies.

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In summary, unraveling the molecular mechanisms underlying all aspects of cancer and better patient stratification will aid the understanding of the unique characteristics of an individual tumor and will help to turn cancer from a devastating deadly disease into a chronic disease. Technologic developments in the field will continue to enable discovery research and speed of the translational process. Therefore, the "2nd Kloster Seeon Meeting on Mouse Models of Human Cancer" will accordingly be held in May 2016.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Grant Support

This work was financially supported by the Kloster Seeon Meetings "Mouse Models of Human Cancer" and the nationwide mouse tumor model consortium "Preclinical Comprehensive Cancer Center" (www.helmholtz-pccc.de) through the Helmholtz Association of German Research Centers (Berlin) and the German Cancer Research Center DKFZ (Heidelberg).

Received May 13, 2014; revised June 20, 2014; accepted June 23, 2014; published OnlineFirst August 18, 2014.

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