

MOUSE MODELS FOR CANCER

The mouse is used as a model organism for establishing the role of oncogenes and tumor suppressor genes in tumor development. By utilizing Cre/Lox mediated switching and taking advantage of somatic gene transfer methods, expression of multiple oncogenes and tumor suppressor genes can be regulated in a tissue-specific and spatial-temporal fashion. This permits a more accurate modeling of tumorigenesis as it occurs in man. It provides the opportunity to carefully define relevant genotype-phenotype correlations. The models also permit us to identify new oncogenes and tumor suppressor genes involved in tumor progression using a variety of techniques, such as array CGH, expression profiling and proviral insertional mutagenesis. Finally, these models constitute an excellent experimental system to test prevention and intervention strategies.

Functional analysis of oncogenes and tumor suppressor genes We utilize mice carrying combinations of different oncogene and conditional tumor suppressor gene alleles to model a range of tumors. Our current focus is on several lung cancer subtypes and mesotheliomas. To achieve (sporadic) activation of oncogenes and inactivation of tumor suppressor genes we use Adeno-Cre or Lentivirus-mediated somatic gene transfer to switch the conditional oncogenes and tumor suppressor gene alleles in the desired tissues. Subsequently, tumor initiation and progression is monitored in longitudinal studies using noninvasive imaging techniques.

Lung tumors We focus on small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). When *Rb* and *p53* are inactivated specifically in lung, SCLC develops in nearly 100% of the mice. The marker profile of these tumors closely resembles that of human SCLC. Even similar genomic aberrations are found such as the amplification of the *L-Myc* gene. These tumors are often heterogeneous consisting of different cell types that either grow as spheres in suspension or attached to substrate. Cells growing in suspension carry neuroendocrine markers whereas the adherent non-neuroendocrine cells show more progenitor-like features. Interestingly, these phenotypically very diverse cell lines share distinct genetic aberrations indicating that they were derived from a common progenitor. We wondered why these different cell types persisted in the tumor. Subcutaneous grafting each of the cell types independently gave rise to localized tumors that retained the features of the inoculated cells. However, grafting mixtures resulted in local growth as well as metastasis of the neuroendocrine cells to liver indicating that the non-neuroendocrine cells in the graft empowered the neuroendocrine cells to metastasize. Interestingly, mutant *Hras* can convert the neuroendocrine cells into non-neuroendocrine cells. In spite of an intensive effort we have not yet identified the underlying mechanism that enables the neuroendocrine tumor cells to form metastases.

There is increasing interest in the cell of origin of a tumor as this is likely a key factor in determining the behavior of the tumor with respect to malignant progression and response to therapy. To gain insight into the cell of origin of SCLC and NSCLC we have designed a series of cell-type specific Adeno-Cre viruses that enable us to switch oncogenes and tumor suppressor genes in distinct lung cell types in vivo. We equipped adenoviruses with specific promoter constructs that would upon infection of lung cells drive Cre expression specifically in Clara cells, Alveolar type II cells, neuroendocrine cells or basal epithelial cells. In this way we can assess which cell type can give rise to which tumor type and which oncogenes and tumor suppressor gene mutations are needed catalyze this process. It appeared that Cre driven from a neuroendocrine-specific promoter in conditional *Rb/p53* mutant mice gives rise to SCLC with high efficiency, whereas Cre driven from the alveolar-specific promoter SPC shows a much lower incidence and delayed onset of SCLC. Expression from a Clara cell-specific promoter caused mostly hyperplasias in the bronchial epithelial lining and resulted only rarely to SCLC. When similar experiments are performed in conditional mutant *KrasG12D* mice NSCLC is most efficiently induced with AdenoCre carrying an alveolar type II specific promoter. Interestingly, Clara cell specific expression resulted in tumors with different features indicating that the cell of origin is an important factor for the tumor that arises.



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Publications

Calbo J, van Montfort E, Proost N, van Drunen E, Beverloo H, Meuwissen R, Berns A. *A functional role for tumor cell heterogeneity in a mouse model of Small Cell Lung Cancer. Cancer Cell* 2010 (in press)

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Mesotheliomas Previously we have shown that inactivation of *Nf2* and *Ink4a/Arf* or *Nf2/p53/Ink4a* by intrathoracic Adeno-Cre injection of compound conditional knockout mice gives rise to mostly sarcomatoid mesotheliomas and that *Ink4a* plays an important role in the aggressiveness of the tumor. Also germline *Ink4b/Ink4a/Arf* triple knockout mice show a high incidence of mesotheliomas. Furthermore, sporadic local inactivation of these genes in combination with activation of mutant *KiRas* results in mesotheliomas with an epitheloid phenotype, closely resembling the predominant mesothelioma subtype found in man. We have made this model more versatile. First, we use normal mesothelial cells isolated from the various mutant mice as a starting point. These cells can be propagated in vitro. This not only permits efficient in vitro switching of conditional alleles but also easily allows introduction of other genes or shRNA constructs. Subsequent injection of these cells in recipient mice gives rise to mesotheliomas. This permits us to test responses to specific inhibitors both in vitro and in vivo. Interestingly, we noted that also in this system different cells of origin appear to give rise to different mesothelioma subtypes, each with a different response profile to the various drugs. We will focus on specific resistance mechanisms underlying these differences. This is particularly interesting since these differences are not caused by specific mutations but by the characteristics of the cell of origin.

Parallel to these experiments we have begun to establish cultures from mesothelioma specimen of human mesotheliomas. So far it has been difficult to achieve growth after grafting in immunodeficient mice. We are continuing these experiments now with *Nod;commonGammaKO;Rag2KO* mice.

Ink4 proteins We have produced *Ink4*-less mice. Mice lacking all the four *Ink4* genes are viable but specific compound *Ink4* mutants show a higher incidence of distinct tumors. We are currently making a complete inventory of increased susceptibilities of the different strains and will focus on specific tumor types in which loss of multiple *Ink4* alleles likely play an important role.

ES cell lines from compound mutant mice We have also invested in the development of methods to generate swiftly complex compound mutant mice. The concept is to re-derive embryonic stem (ES) cells from available compound mutant strains and use these ES cells to introduce additional genetic changes by gene targeting or the exchange of expression cassettes. This approach has become within reach by the description of defined media (2i and 3i) that prevent the differentiation of ES cells. Using this approach we have successfully derived a series of ES cell lines from the strains we routinely use. Injection of these ES cells in pre-implantation embryos results in the majority of cases in chimeras with extensive ES cell contribution. We are testing whether these chimeras can be directly used for tumorigenesis studies. If this is the case we can quickly assess the relevance of distinct genetic alterations on tumor incidence and tumor characteristics without having to embark on lengthy and extensive breeding protocols. It also would substantially reduce the number of mice needed for most of our tumor studies.