Abstract 646

# Identification of FURIN as a tumor maintenance gene in solid tumors by *in vivo* Retroviral Mutagenesis and Directed Complementation

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## Abstract

To identify tumor maintenance genes and pathways, we applied a genome-wide, large-scale functional screening approach using retrovirus-mediated mutagenesis on a number of inducible murine solid tumor models, including a HER2-driven breast tumor model and kRAS-driven breast and lung cancer models, in which viral integration could maintain established tumors in the absence of the initiating oncogene.

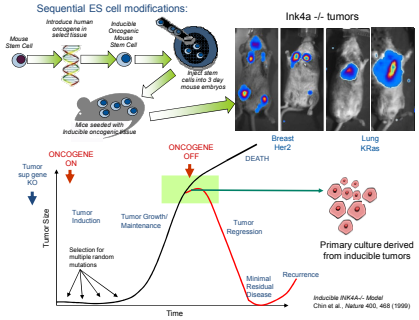
We identified more than 3,000 recurrent viral integration sites in a collection of about 400 tumors. Whereas some genes were targeted by viral integration in specific tumor models, others were prominently hit in all the models. For example, insertions targeting the *Furin* locus were detected in tumors from all three models. In such tumors, viral integrations mapped to the *Furin* promoter consistent with the notion that activation of this gene and/or pathway may represent a common mechanism of tumor maintenance.

Using our inducible directed complementation (DC) platform, we show that introduction of a wild type FURIN cDNA can maintain primary tumors *in vivo* in the absence of the initial driving oncogene. The FURIN DC tumors provide a useful pre-clinical model with which we can examine the effects of FURIN antagonists on tumor maintenance. Next, we sought to extend the utility of these validated DC tumor models to drug discovery by establishing FURIN-dependent cell lines for high-throughput screening.

Here we describe detailed molecular characterization of the FURIN DC tumor models as well as from the cell lines derived from these tumor models. Furthermore, FURIN DC Tumor Models as wells as cell lines serve as a unique experimental tools, with which response to novel targeted therapies and other cancer agents can be studied.

## Inducible Tumor Models

Complex Models Can Be Generated Rapidly
Inducible Breast and Lung Tumors (Kras, HER2)




## Insertional Mutagenesis Genetic Screens in AVEO 's Inducible Tumor Models

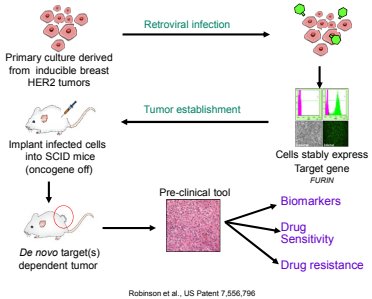
*In vivo* Moloney insertional mutagenesis screens in multiple inducible tumor models


- Comprehensive, large-scale, genetic screens were conducted to identify candidate tumor maintenance genes using AVEO's inducible tumor models.
- These screens identified well-known, as well as many unexpected cancer targets not previously known to be related to tumor maintenance.


## Generation of FURIN Tumor Models

Directed Complementation Technology

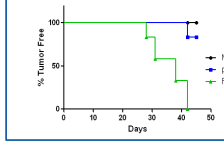
- Creation of tumors driven by chosen target (directed complementation):
  - Allows for study of target biology in an *in vivo* tumor context
  - Allows for correlation between mutational variants and drug response



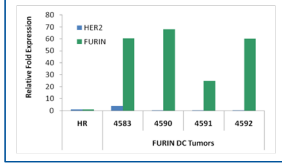

Robinson et al., US Patent 7,556,796

## FURIN DC Tumor Models

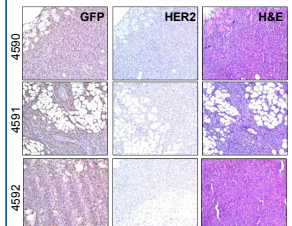
**Latency and Penetrance of FURIN DC Tumor Models**



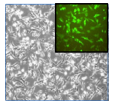

**FURIN DC Tumors Express high levels of FURIN and low levels of HER2 (RT-PCR)**



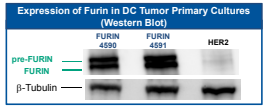

**FURIN DC Tumors Express high levels of GFP and no HER2 (IHC)**



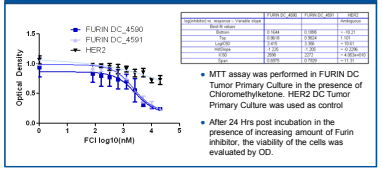

## Generation and Validation of FURIN DC Tumor Primary Cultures

**Expression of Furin in DC Tumor Primary Cultures (Western Blot)**

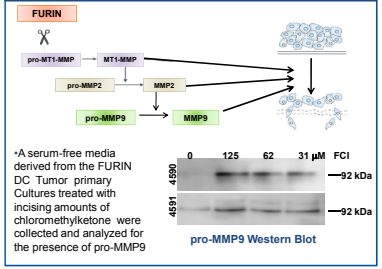



**FURIN DC Tumor Primary Cultures are sensitive to a Furin Convertase Inhibitor (Chloromethylketone)**



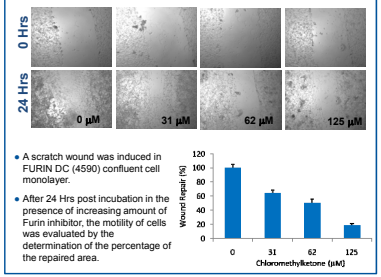

- MTT assay was performed in FURIN DC Tumor Primary Culture in the presence of Chloromethylketone. HER2 DC Tumor Primary Culture was used as control
- After 24 Hrs post incubation in the presence of increasing amount of Furin inhibitor, the viability of the cells was evaluated by OD.

**Endoprotease Activity in FURIN DC Tumor Primary Cultures**




•A serum-free media derived from the FURIN DC Tumor primary Cultures treated with incising amounts of chloromethylketone were collected and analyzed for the presence of pro-MMP9

**Regulation of FURIN DC Tumor Primary Culture cell motility by Furin Convertase Inhibitor (Chloromethylketone)**




- A scratch wound was induced in FURIN DC (4590) confluent cell monolayer.
- After 24 Hrs post incubation in the presence of increasing amount of Furin inhibitor, the motility of cells was evaluated by the determination of the percentage of the repaired area.

## Summary

- **Comprehensive, large-scale, genetic screens were conducted to identify candidate tumor maintenance genes in multiple AVEO's inducible Tumor Models. Several tumors carried retroviral insertions targeting the endogenous *Furin* locus.**
- **Introduction of defined cDNAs into inducible tumor background (Directed Complementation or DC) allows for creation of target-dependent tumor models. Using this approach, we created FURIN-directed complemented tumor models, validating this endoproteinase as a tumor maintenance candidate target.**
- **Cell primary cultures were established from FURIN DC Tumor Models which show sensitive to Chloromethylketone treatment, a well established Furin Convertase Inhibitor (FCI).**
- **Furin endoprotease activity and its role in cell motility were measured in FURIN DC Tumor Primary Cultures in the presence of FCI.**
- **Together both in vivo and in vitro models provide ideal preclinical settings for the development of targeted anti-FURIN therapies, as well as a tool for dissecting their accompanying molecular correlates of response.**

**Acknowledgments**
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