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

Lorena Lerner, Qing Liu, John Yang, Nianjun Tao, Brian Krieger, Jodi Zarycki*, Fanglei You*, Omar Kabbarah*, Murray O. Robinson, and M. Isabel Chiu

AVEO Pharmaceuticals, Inc., Cambridge, MA, USA (* Former AVEO employees)

Abstract

To identify tumor maintenance genes and pathways, we applied a genome-wide, large-scale functional screening approach using retroviral-mediated mutagenesis in a number of inducible mouse solid tumor models, including a large number of ONCOGENE-dependent cell lines for high-throughput screening. Using this approach, we created FURIN-directed complemented tumor models, in which viral integration could maintain established tumors in the absence of the initiating oncogene.

We identified more than 2,000 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 the Gamma 13/14 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 tumor models provide an inducible model system in which the expression of endogenous FURIN can be regulated as an oncogene. Next, we sought to extend the utility of these validated DC tumor models to drug discovery by establishing FURIN-directed complemented tumor models, validating this endoproteinase as a tumor maintenance candidate target.

Summary

- 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 related to tumor maintenance.
- 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 culture models were established from FURIN DC Tumor Models, which allows 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

Study supported by AVEO Pharmaceuticals, Inc., Cambridge, MA.