Isoform-Specific Targeting of Cancer-Associated Genes by Small Interfering RNA in Ovarian Carcinoma

Lin Zhang, George Coukos

Center for Research on Reproduction and Women’s Health
Abramson Family Cancer Research Institute
University of Pennsylvania School of Medicine
RNA interference (RNAi), a sequence-specific post-transcriptional gene silencing mechanism.
Exon-Specific RNAi

- The majority of metazoan genes encode pre-mRNAs that are subject to alternative splicing.
- As many as 74% of human genes encode alternatively spliced mRNA.
- An alternative spliced gene can generate anywhere from 2 to 38,016 different isoforms.
- Different protein isoforms synthesized from a single gene have distinct functions.
- Multiple isoforms of a large percentage of human proteins associated with cancer are produced by alternative RNA splicing.
- Isoform-specific therapeutic method is very limited.
**Vascular Endothelial Growth Factor (VEGF) in Ovarian Carcinoma**

**Human ovarian cancer**

VEGF is associated with poor outcome of ovarian carcinoma.

- VEGF promotes tumor angiogenesis;
- VEGF suppresses anti-tumor immune response;
- VEGF exerts autocrine function on tumor cells.


**Murine ovarian cancer model**


VEGF exists as at least five isoforms produced by alternative splicing. Various VEGF isoforms may perform distinct functions as well as cooperate with each other in tumor development.

E.g. larger molecular weight isoforms are associated with poor outcome in some solid tumors.
Isoform-specific RNAi and qPCR detection methods

- mVEGF188
- mVEGF164
- mVEGF120

∞ siRNA

∞ probe  ∞ primer
RNAi specifically knocked-down mVEGF isoforms in ID8 cells

A: siVEGFcom
GCUACUGCGUCCAAUUGA
CGAUGACGCGAGGUUAACU
U

B: siVEGF164/188
GGCGAGGCGAGCUUGAAGUA
CAGCUCCGUGCACUCAAU
U

C: siVEGF188
AAUCAGAUUGGAAAGGG
UAAGUCAAGCUCCUUCCTC
U
Suppressed gene expression by RNAi is highly target sequence-specific.
Phosphatidylinositol 3'-kinase catalytic subunit alpha (PIK3CA) is an oncogene in Ovarian Carcinoma

RNAi knocked down PIK3CA expression and decreased tumor cell proliferation

Specificity of PIK3CA RNAi targeting
Efficient delivery systems need to be developed.

Tissue type and/or cancer specific siRNA strategy needs to be developed.
Acknowledgments

The George Coukos Lab

Collaborators

University of Turin, Italy
Dionyssios Katsaros
Strfano Fracchioli
Marco Massobrio

Material Contributors

Patricia D’Amore    Harvard University
Paul F. Terranova    University of Kansas
Warren Pear    University of Pennsylvania

Core Service of CRRWH
DNA Core
Microdissection Core

[Image of a group of people]