Genomically guided discovery of cardiovascular therapeutics

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"Hard" facts about atherosclerosis
Trichrome stain of diseased left anterior descending artery

• 3,000 heart attacks/day in U.S.
• 50% of first attacks are fatal
• at ER, almost 90% survive
• >50% of victims have no overt risk factors
• about twice as likely to die of heart disease as cancer
• if diagnosed, coronary disease is very treatable

“We’ll widen the clogged artery by inserting a balloon.”

Endovascular Stents

About 650,000 patients per year have stents placed in the US alone.
Angioplasty of coronary arteries

Before stent placement  After stent placement

Courtesy Jon Reiner, M.D., MFA/GW-Cheney Cardiovascular Instit.

Base-pairing

Sample in SOLUTION

Microarray Production Facility

DNA synthesizer

HEPA-filtered clean room

Magnified slide area

In-Stent Restenosis

LUMEN

STENT

Purines

Pyrimidines

SOLID GLASS SURFACE

Base-pairing

Figure 1.4. The nucleic acid base-pairing is driven by hydrogen bond formation between complementary bases. The complementary bases are adenine (A) and thymine (T), and guanine (G) and cytosine (C).
Photolithographic Synthesis

“Etching” DNA sequences

GeneChip® Probe Array

GeneChip® Probe Arrays
Data Flow Overview

Chip Image → Probe Intensities and Detection Calls → Gene List and Heat Map

GeneChip analysis of human atherosclerosis

Dissect normal media from atherosclerotic lesion

Prepare highly purified RNA

O.D. 260/280 = 2.0

Reverse transcribe w/poly dT + T7 = cDNA

Transcribe with T7 + biotin dUTP = cRNA

Purify probe/hybridize to chip

Compute levels and compare.

Typical scatter plot of results

Interferon-Treated human vascular cells

(X-axis: Interferon GC-RMA (Default Interpretation) : INF(3) con

Y-axis: Interferon GC-RMA (Default Interpretation) : INF(3) inf

Colored by: Interferon GC-RMA, Default Interpretation (INF(3) inf )

Gene List: all genes (22283)

10 100 1000 1e4

INF(3) con  (normalized)
Pathway Analysis
Lists of differentially expressed genes are compared to curated networks

Bcl-Xl overexpression in stable lines

Bcl-Xl strongly confers resistance.
Western blot after fas ligation by CH11 antibody

Bcl-Xl blocks initiator caspase activation.
Western blot after fas ligation by CH11 antibody
Small molecule Bcl-2/XL inhibitors

Surface model of Bcl-XL

Bcl-2/XL inhibitors induce sensitivity to fas ligation.
Competitive reversal by Bcl-XL overexpression

Apoptotic resistance in lesion cells

Genomic ‘promise’.

Diagnosis of disease
- high risk individuals can be identified for monitoring
- single disease is separated into different genetic causes

Predict drug responses
- FDA near approval on test for codeine efficacy
- genetic tests allow drugs with low risk of side effect

Identify new targets for drug therapy
- compare normal vs. disease tissue identifies cause(s)
Engineering issues:

- 'cloud' or distributed computing
- reusable microarrays
- electrochemical sensing?
- acoustic sensing of arterial turbulence

Collaborators

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"Either this is the wrong chart or—let's just hope this is the wrong chart."