Science on the NIH Road Map: The Center for Nutrient-Gene Interaction (CNGI) in Cancer Prevention

Stephen Barnes, PhD
Professor of Pharmacology and Toxicology and CNGI Director
UAB
Northern California Cancer Center
U. Penn
U. Missouri-Rolla

A multi-disciplinary multi-institutional center
Synopsis of this talk

• NIH and its beginnings - development of the research paradigm
• Cancer research - the search for the cure
• Living with estrogens (and polyphenols)
• Cancer Research - the shift to prevention - a role for CNGI
• How is the NIH research paradigm changing?
Origins of NIH research

1930 - Ransdell Act establishes the National Institute of Health
1937 - National Cancer Institute established
1948 - Four new institutes (cardiac disease, dental disorders, infectious diseases, and experimental biology and medicine) and a Clinical Research Center leads to the *National Institutes of Health*
1971 - National Cancer Act
1990 - Human Genome project
1998 - Twenty seven institutes and centers constitute the National Institutes of Health

CNGI Seminar 11-19-03
Origins of Federal Biomedical Research

National Institutes of Health, 1949

- Federal Security Agency
- Public Health Service
- National Institutes of Health, 1948
  - National Cancer Institute, 1937
  - Division of Research Grants, 1946
  - National Heart Institute, 1948
  - National Microbiological Institute, 1948
  - Experimental Biology and Medicine Institute, 1948
  - National Institute of Dental Research, 1948
  - National Institute of Mental Health - 1946 (authorized) 1949 (funded)

CNGI Seminar 11-19-03
NIH funding

1937  National Cancer Institute starts grants and fellowship program
1946  Extended to NIH

1947  $4 million
1957  $100 million
1966  $1 billion
1990  $8.5 billion
2002  $23.3 billion
NIH research is linear

• Universities have been increasingly dependent on their development on NIH funding since 1980 (Nature)
• Has been determined by Study Sections
  – Approve what they in their narrow view consider the “best science”
  – Most data and least controversy wins
  – Until 2003, organized according to major scientific divisions
  – In 2003, reorganized around organ systems
Direction of NIH research for the past 50 years

Metabolism → Lipid chemistry → Protein chemistry → Molecular Biology
Cancer Research - the search for therapy

- The immense amount of research funding over the past 60-70 years has been focused on treating the patient who has presented with cancer.

- There have been successes - some cancers have been substantially reduced.

- Stomach cancer is down 90% since the 1930’s, but it was due a preventive policy.
Origins of CNGI at UAB

- 1985  Recruitment of Clint Grubbs
- 1986  Recruitment of Coral Lamartiniere
- 1987  American Cancer Society fund first soy and breast cancer prevention grant (SB/CG)
- 1988  American Institute for Cancer Research continue soy funding (SB/CG)
- 1994  NCI fund an integrated research program grant (IRPG) on genistein and breast cancer (SB/CAL/HW)
- 1997  NCI renew IRPG (SB/CAL)
- 1999-2003 Many USAMRD and NIEHS prevention grants to CAL
- 2000  Botanicals Center for Age-related Disease
- 2003  NCI fund CNGI in cancer prevention
Can cancer be prevented?

- Public health policy has had success in lowering the incidence and death from stomach cancer.
- Control of smoking in public places may also reduce the lung cancer rates.
- What are the events that increase cancer?
  - Environmental chemicals in air, water and food.
  - Endogenous compounds that are altered by social habits.
Estrogens
do they define you, me and cancer?

• We are exposed to high estrogen levels during gestation, during puberty, and during the early part of adult life

• Toxicologists are passionate about defining so-called endocrine disruptors
  – compounds that appear in the environment that have steroid-like activity (contraceptives and other therapeutics that are flushed down the toilet)
  – estrogenic chemicals produced by the chemical industry
  – estrogens in foods
Timeline for estrogen exposure and breast cancer

- Gestation
- Neonatal
- Puberty
- Adult <50 yr
- Adult >50 yr
- Pregnancy
- Contraception
- HRT
- Cancer risk
Effects of estrogen on breast cancer risk at different ages

- Gestational - genistein allegedly pro-cancer
- Neonatal - DES and genistein prevent cancer
- Pre-pubertal - estradiol/progesterone strong prevention, genistein weaker
- Pregnancy - mixed (increased early, less in later years)
- Contraceptive - mixed
- HRT - 2% increased risk per year of use

Summary: estrogens have complex effects
Puberty is a crucial period for estrogen exposure

- The early part of puberty is associated with mammary epithelial cell proliferation
  - See work of Russos’ and Lamartiniere groups
- This is followed by differentiation of the terminal endbuds
- Differentiation limits the number of cells containing damage to DNA
- Estrogen exposure at this stage may also control estrogen responsiveness in adult life
- Are related compounds capable of altering responsiveness?
Genistein is an estrogen in the rat mammary

Cotroneo et al., Carcinogenesis 23:1467, 2002

CNGI Seminar 11-19-03
Early genistein exposure is essential for cancer prevention.

Dietary genistein in adult life is only effective if the animals were given genistein during the prepubertal period.

Lamartinere et al., J Nutr 132:552S
Epidemiologic evidence in support of pubertal prevention in Asia-Americans

<table>
<thead>
<tr>
<th>Adolescent</th>
<th>Adult</th>
<th>Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>Low</td>
<td>1.00</td>
</tr>
<tr>
<td>Low</td>
<td>High</td>
<td>0.93 (0.58-1.48)</td>
</tr>
<tr>
<td>High</td>
<td>Low</td>
<td>0.77 (0.51-1.16)</td>
</tr>
<tr>
<td>High</td>
<td>High</td>
<td>0.53 (0.36-0.78)</td>
</tr>
</tbody>
</table>

P trend 0.001

Wu et al., Carcinogenesis 23:1491 (2002)
# Soy in adolescence and Shanghai breast cancer study

<table>
<thead>
<tr>
<th>Food</th>
<th>Q1</th>
<th>Q2</th>
<th>Q3</th>
<th>Q4</th>
<th>Q5</th>
<th>p trend</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Premenopausal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tofu</td>
<td>1.00</td>
<td>0.72</td>
<td>0.87</td>
<td>0.60</td>
<td>0.79</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Not tofu</td>
<td>1.00</td>
<td>0.90</td>
<td>0.76</td>
<td>0.68</td>
<td></td>
<td>0.01</td>
</tr>
<tr>
<td><strong>Postmenopausal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tofu</td>
<td>1.00</td>
<td>1.10</td>
<td>0.89</td>
<td>0.82</td>
<td>0.62</td>
<td>0.01</td>
</tr>
<tr>
<td>Not tofu</td>
<td>1.00</td>
<td>0.77</td>
<td>0.93</td>
<td>0.62</td>
<td></td>
<td>0.05</td>
</tr>
<tr>
<td><strong>Reports from mothers</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tofu</td>
<td>1.00</td>
<td>1.08</td>
<td>0.72</td>
<td>0.52</td>
<td>0.65</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Not tofu</td>
<td>1.10</td>
<td>0.72</td>
<td>0.79</td>
<td>0.44</td>
<td></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Fresh legumes</td>
<td>1.00</td>
<td>0.77</td>
<td>0.92</td>
<td>0.83</td>
<td>0.96</td>
<td>0.82</td>
</tr>
</tbody>
</table>


CNGI Seminar 11-19-03
Polyphenols and disease risk

resveratrol

catechin

quercetin

genistein

CNGI Seminar 11-19-03
Eating habits at age 12
What are the real activities of genistein and other polyphenols?

- Could be estrogens
- What else?
  - Tyrosine kinase inhibitors
  - Numerous other activities
  - DING protein - a possible analog of Dvl in the Wnt signaling pathway
- Future may lie in the use of DNA microarrays
Evolution of the change in linear research

• A substrate to search for one enzyme activity
• A labeled oligonucleotide or cDNA to probe a library
• Combinatorial chemical library with enzyme or receptor
• An agonist or antagonist to probe a gene array to locate genome-wide or pathway-wide targets
• Mixture of agonists or antagonists with gene arrays
• Protein arrays to find protein-protein interactions
The future of NIH research

- genomics
- proteomics
- lipidomics
- metabolomics

CNGI Seminar 11-19-03
Integration for life extension in yeasts

Caloric restriction increases yeast life span - dependent on the SIR-2 gene (a sirtuin) - a NAD⁺-dependent deacetylase - calorie restriction increases NAD⁺

Human equivalent SIRT1 - deacetylates p53 (used K382 peptide)

Sinclair et al. screened a library of compounds - quercetin and piceatannol emerged as activators of deacetylase

![Chemical structures of piceatannol and resveratrol]

Resveratrol increased life span of the yeast by 70% in the absence of caloric restriction, decreased p53 K382 acetylation, and reduced the frequency of repetitive DNA recombination
Technologies favored by the NIH roadmap

• Molecular Libraries and Molecular Imaging
• Bioinformatics and Computational Biology
• Nanomedicine
• Structural Biology
• Building Blocks and Pathways
  Gene arrays
  Proteomics
  Metabolomics/metabonomics
Protein analysis 2003

Image analysis → Robotic spot picking → Destaining, drying and trypsinolysis

MALDI plate

Desalting Ziptip
Tryptic mass fingerprint of a porin from *Drosophila*

1181.59 YQLDDDASVR

1 MAPPSYSDLG KQARDIFSKG YNFGLWKLDL KTKTSSGIEF NTAGHSNQES
51 GKVFGSLETK YVKDYGLTL TEKWNTDNTL FTEAVQDQL LEGLKLSEG
101 NFAPQSGKGN GKFKVAYGHE NVKADSDVNI DLKGPLINAS AVLGYQGWLA
151 GYQTAFDTQQ SKLTTNNFAL GYTTKDFVHL TAVNDGQEQS GSIFQRTSDK
201 LDVGVQLSWA SGTSNKFAI GAKYQLDDDA SVRAKVNNAS QVGLGYQQKL
251 RDGVTTLTST LVDGKKNFAG GHKIGVGLEL EAA

1443.76 LTTNNFALGYTTK
1461.75 LSLEGNFAPQSGNK
1950.89 TSSGIEFNTAGHSNQESGK

1016.55
1034.54
1184.60
1583.79
1756.86
2164.05 trypsin autolysis product
2185.02
2368.13
2813.32
Unexpected peptide from a bacterially expressed protein

MELEMQLTATPVSALVDEPVHIRE
Understanding the protein-protein network

Mapping the protein:protein network by antibody or affinity isolation

Multi-protein complex

CNGI Seminar 11-19-03
Summary

• The NIH Roadmap represents a challenge to the science community
  – It’s time to cure disease
• The roadmap is supported by all the NIH institutes
• It requires the integration of widely separated skills, particularly in high level computing, statistics and nanotechnology
## 2003 CNGI Pilot Program

<table>
<thead>
<tr>
<th><strong>Deadline:</strong></th>
<th>December 9th, 2003</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Format:</strong></td>
<td>NIH 398 with a 10-page restriction for sections A-D</td>
</tr>
<tr>
<td><strong>Deliver to:</strong></td>
<td>454 McCallum or by e-mail to <a href="mailto:Rose.Johnson@ccc.uab.edu">Rose.Johnson@ccc.uab.edu</a></td>
</tr>
<tr>
<td><strong>Topic:</strong></td>
<td>Dietary polyphenols, a cell or multi-cellular organism model that responds to steroids, pathway exploration</td>
</tr>
<tr>
<td><strong>Other:</strong></td>
<td>Use of genomics, proteomics, and/or statistics cores</td>
</tr>
<tr>
<td><strong>Award:</strong></td>
<td>$25,000-$40,000 (3-5 awards)</td>
</tr>
</tbody>
</table>
Principal hypotheses in UAB CNGI

• That the set points of expression of genes that are under the control of steroid hormones during puberty are strongly associated with adult cancer risk (Lamartiniere)

• That the set points are controlled both by the polymorphisms of the genes and by dietary polyphenols (Horn-Ross, NCCC)

• And that extracting the answers from high dimensional data requires the discovery and implementation of novel statistical and computing techniques (Allison)
Core Support in UAB CNGI

• Administration (*Barnes/Grubbs*)

• DNA analysis: DNA microarrays and gene polymorphisms (*Guay-Woodford*)

• Proteomics and mass spectrometry (*Kim/Barnes*)

• Biostatistics and Bioinformatics
  (*Soong/Chen/Desmond/Hill/Lefkowitz/Meleth/Page*)
Acknowledgments

Coral Lamartiniere
Pam Horn-Ross
David Allison
Clint Grubbs
John Milner, NCI
Lisa Guay-Woodford
Helen Kim
Seng-Jaw Soong

Leadership of the UAB Comprehensive Cancer Center

John Yates
Kei Cheung
John Quackenbush
Michael Gould
Don Hill
Stuart Frank

Jun Wang
Christine Collins
Grier Page
Jode Edwards
Kathy Scheirer
Marion Kirk
Landon Wilson
Jessy Deshane
Heath McCorkle
Todd Sanderson
Aubrey Hill
Elliott Lefkowitz
Renee Desmond
Sreelatha Meleth
Rose Johnson
Ramu Vempati

Support from NCI U54 CA100929

CNGI Seminar 11-19-03