

Markers for Early Diagnosis and Prevention of Cancer

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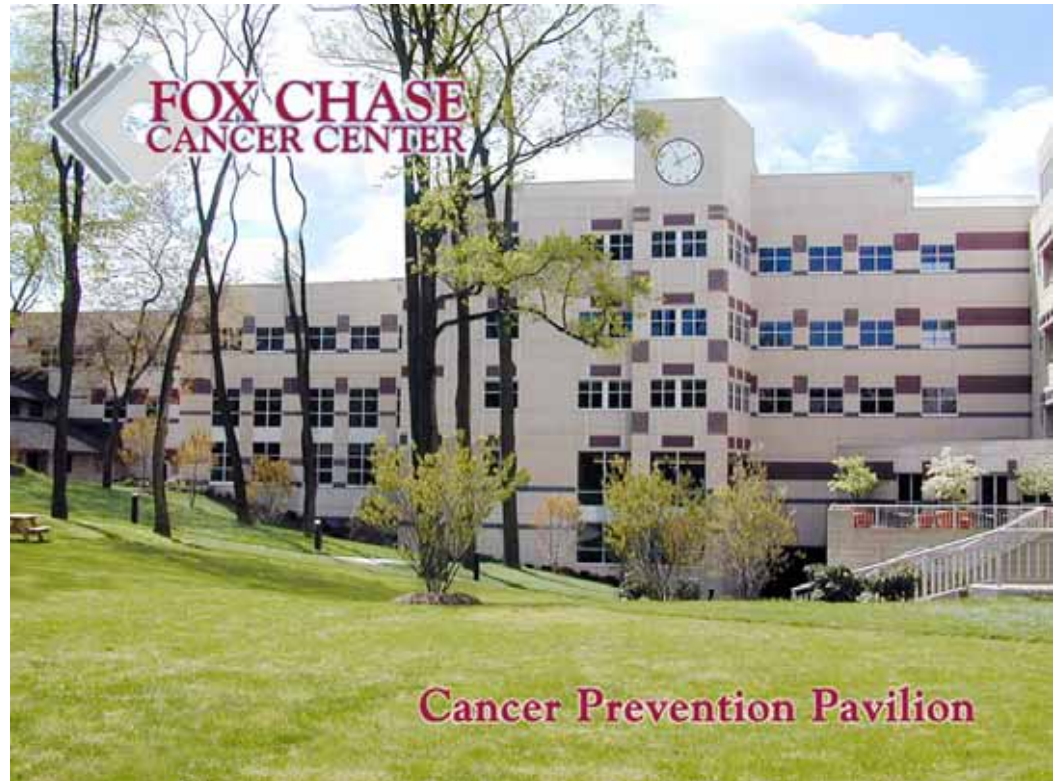
Levy Kopelovich, Jim Crowell
Marston Linehan

Workstatements #37 & #40

Premises

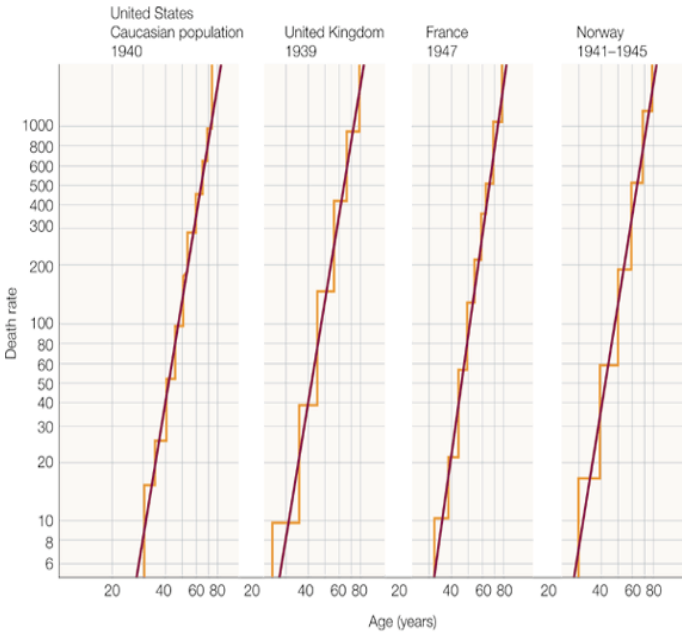
- Some of the best strategies for cancer control include primary prevention and early diagnosis
- There is a compelling need to identify reliable and effective biomarkers of cancer risk for the common malignancies (ca. breast, ovary, colon and kidney)
- Previous approaches to identify reliable biomarkers are based on the use of animal models or require relevant clinical samples (bodily fluids, tissues, etc.). However, animal data are often not translatable to humans, and bodily fluid or human sample analysis can be invasive for the patient.
- Genomic technologies are ripe for biomarker identification

A genomic approach to cancer prevention:
large-scale gene expression profiling
by microarray analysis for the identification
of very early molecular changes



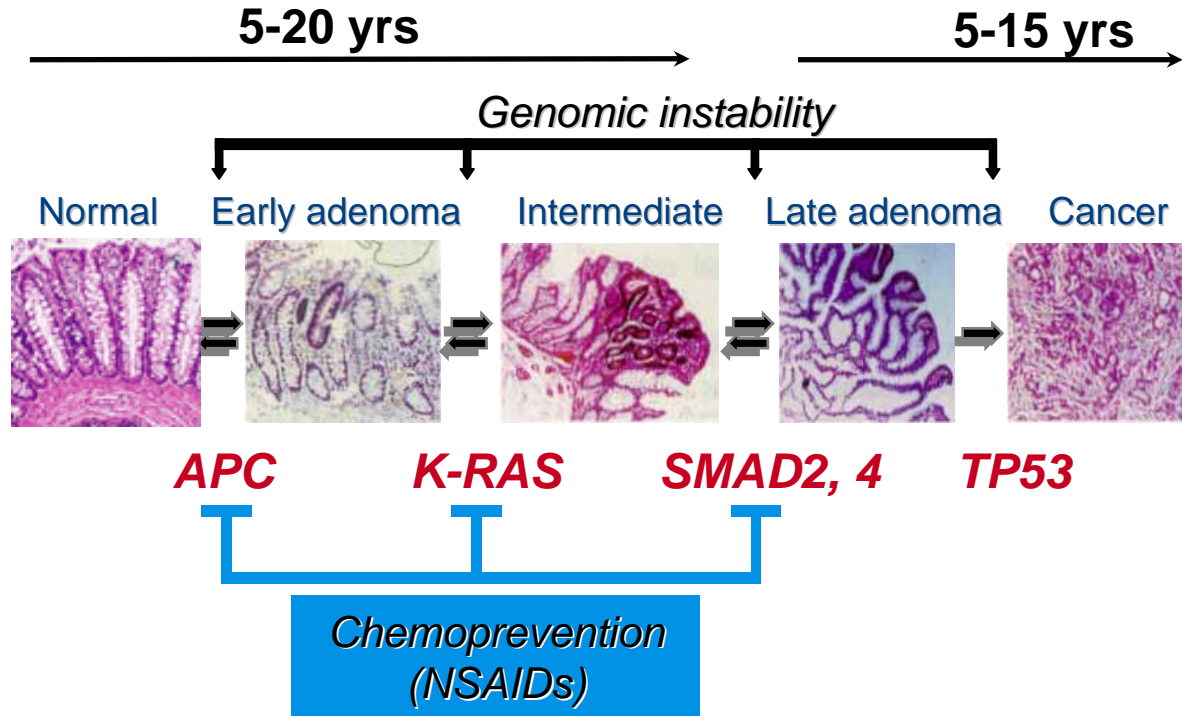
Epidemiology of common epithelial cancers - Multistep Tumorigenesis

$$I = k t^{r-1}$$



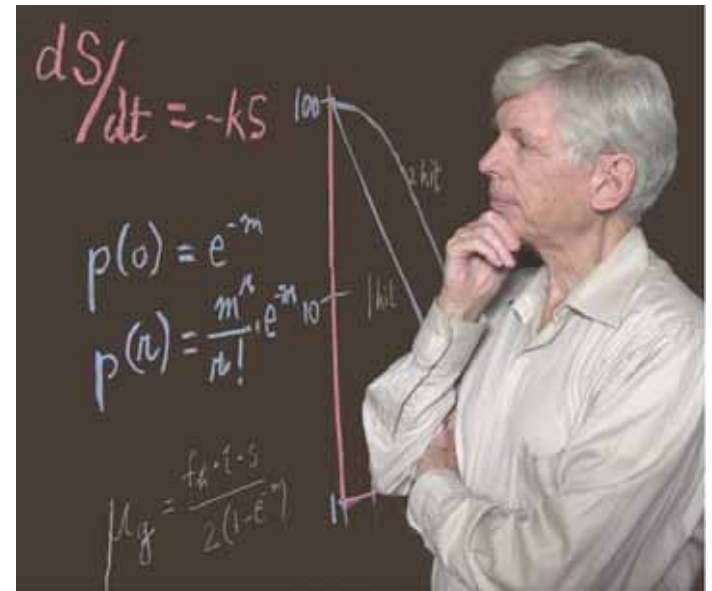
A. Knudson

Nature Reviews | Cancer



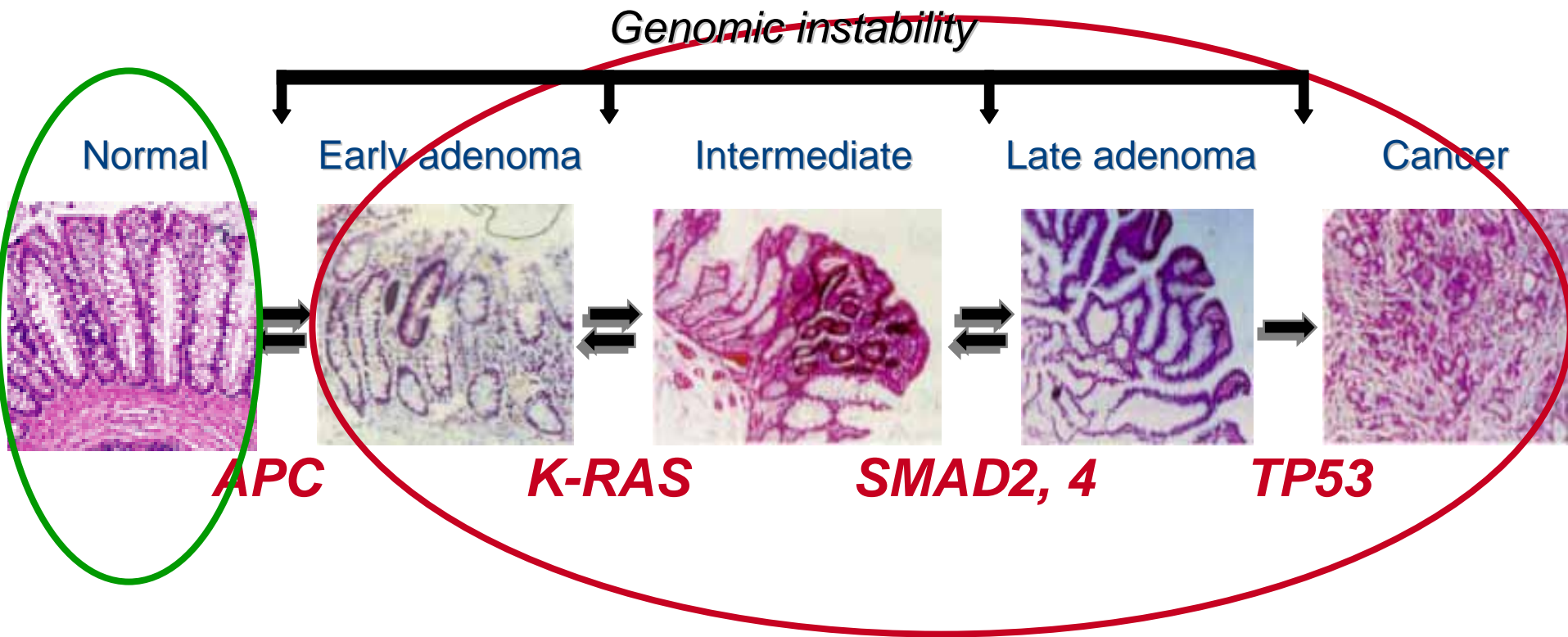
Fearon & Vogelstein
Ilyas et al.
Kelloff et al.

Alfred G. Knudson
Oncology Times (1996)
- War on Cancer -



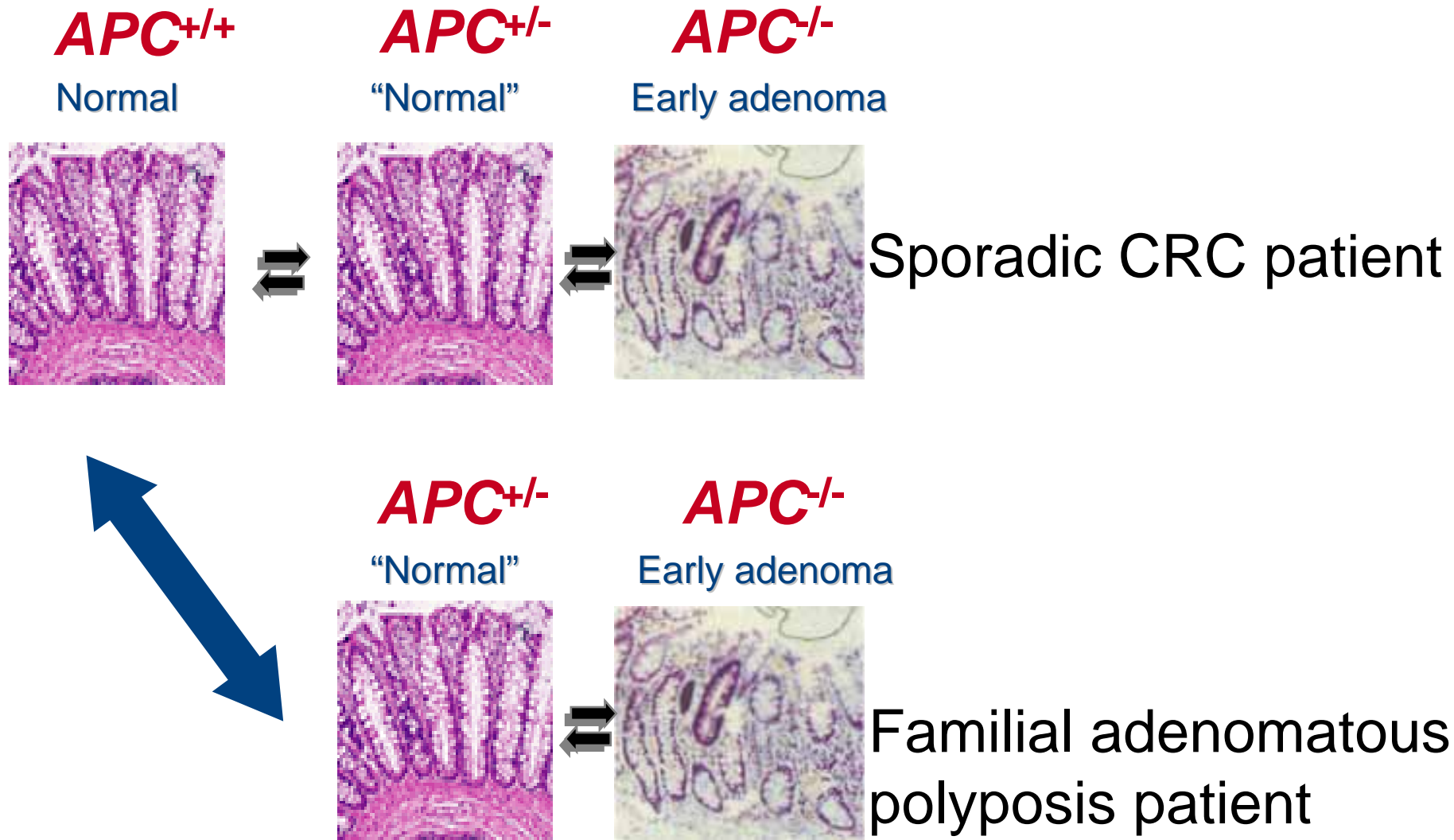
“If a tumor is caused by a very small number of events, it would be very hard to intervene and prevent it from happening, but if it takes several events to make a cancer and we can slow down the rates for these events in a particular target organ, we may be able to prevent the tumor from happening, or shift the age at which an individual gets cancer by 20 or 25 years.”

Pitfalls of conventional microarray analysis of multistep tumorigenesis: normal, adenoma, carcinoma



Research Strategy

A genetic strategy to identify early molecular alterations based on individuals predisposed to cancer (carriers of mutations in tumor suppressor genes)



Workstatement #37 (P.I. A.G. Knudson,
Program Coordinator A. Bellacosa,
Co-Directors M. Clapper & B. Boman)

“Evaluation of *In Vivo* and *In Vitro* Pharmacology and Toxicology of Preventive Agents Using Human Mutant Cells From Dominantly Heritable Cancers”

Hypothesis: Non-tumorous cells from carriers of a first genetic “hit” display the fewest molecular aberrations along the pathway to cancer. The analysis of histologically normal cells from mutation carriers in comparison to controls is expected to highlight the earliest of these molecular aberrations as changes in the mRNA expression profile

Genetic Conditions under Evaluation

Disease	Genotype	Target tissue investigated
FAP	<i>APC</i> *	colon
HNPCC	<i>MLH1</i> ^o	colon
Hereditary breast and ovarian cancer	<i>BRCA1</i> ^o <i>BRCA2</i> ^o	breast / ovary breast / ovary
Tuberous sclerosis	<i>TSC2</i> *	kidney
Von-Hippel Lindau	<i>VHL</i> *	kidney

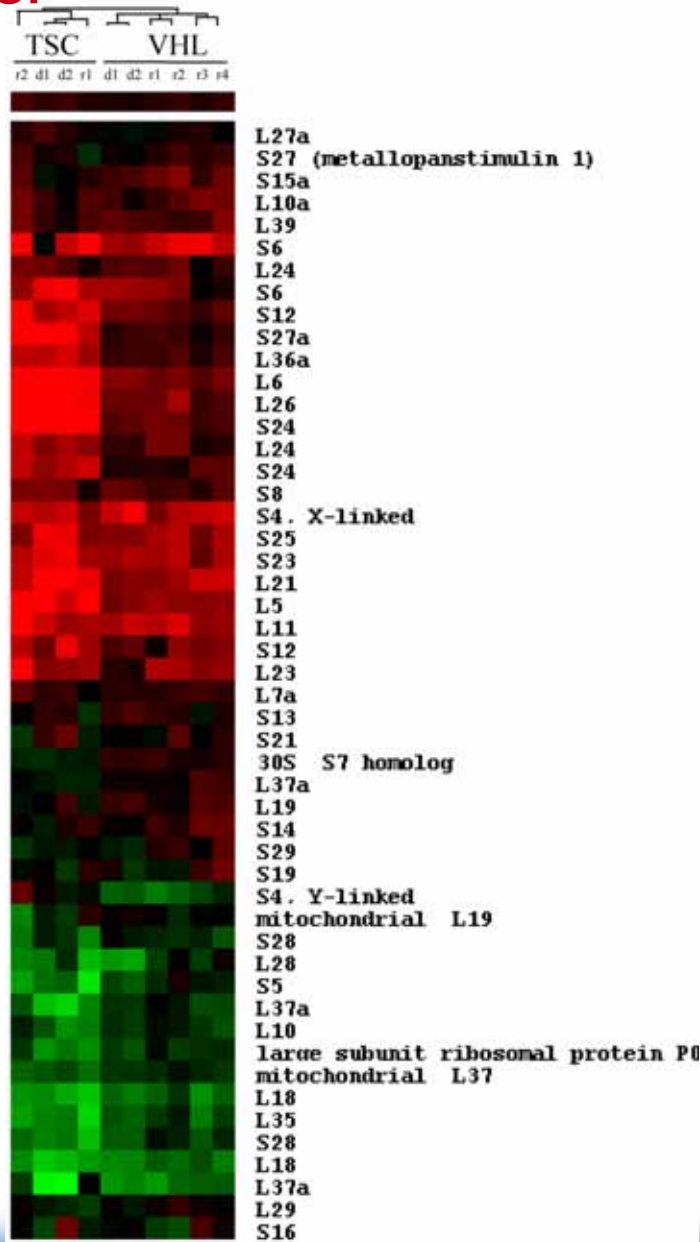
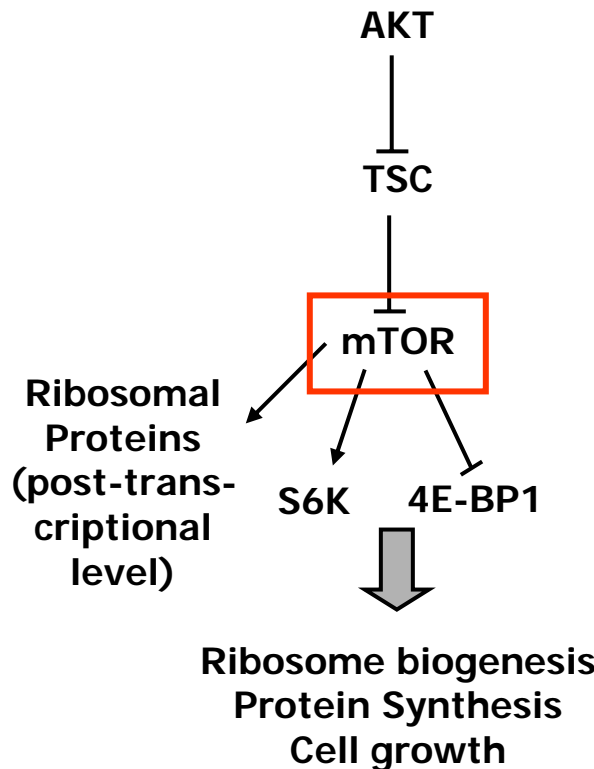
* tumor suppressor gene; ^o DNA repair gene

Kidney cancer

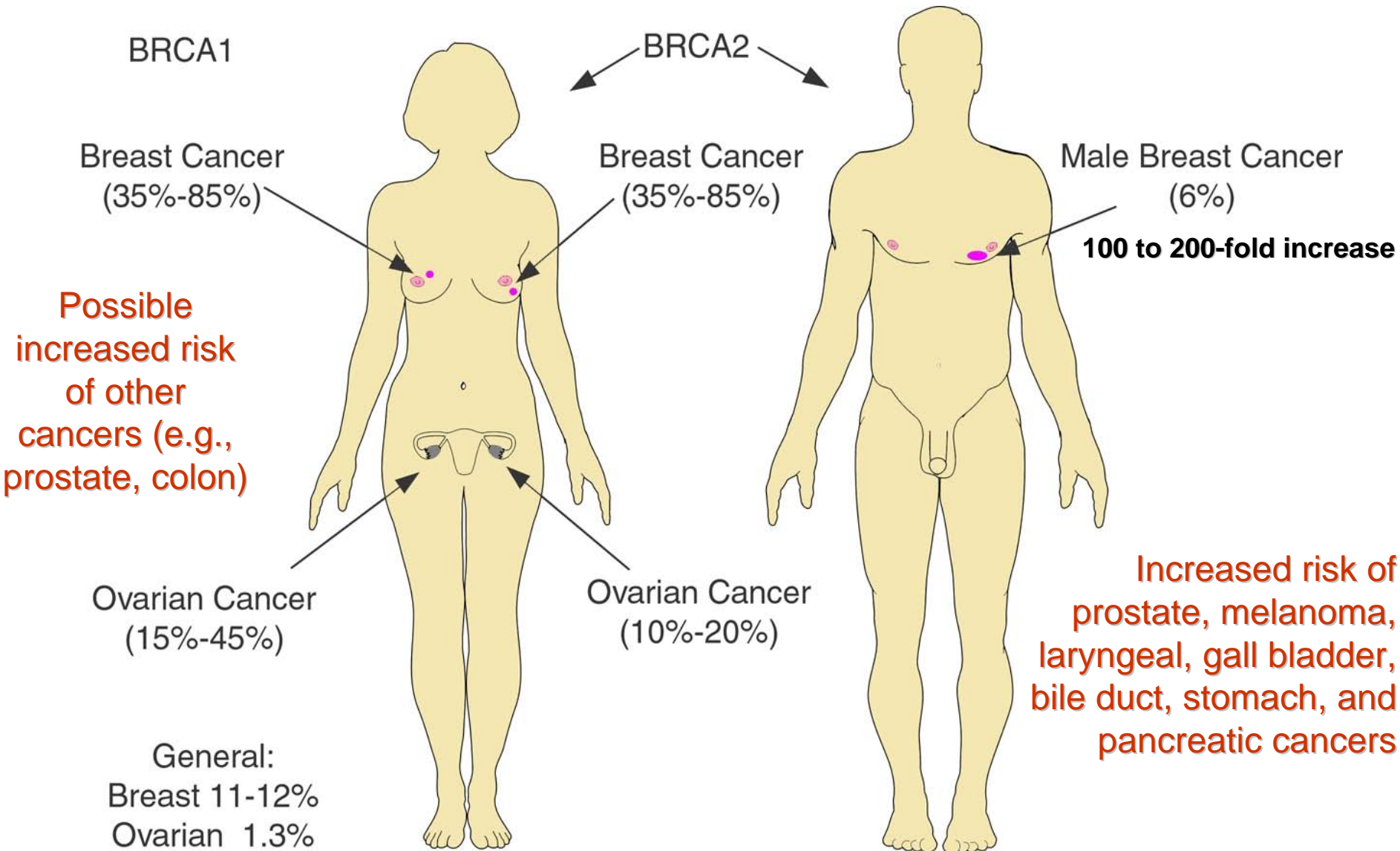
Two models based on mutations of tumor suppressor genes that predispose to renal cancer were used in previous studies:

- **Tuberous sclerosis complex (TSC)** is a rare, multi-system genetic disease that causes benign tumors in the brain, kidneys, hearts, eyes, lungs, and skin. This syndrome is caused by mutations on either the TSC1 (hamartin) or TSC2 (tuberin) gene.
- **Von Hippel-Lindau (VHL) disease** is a rare inherited genetic condition characterized by the presence of numerous tumors originating from blood vessels (especially in the retina, spinal cord, and cerebellum).

Phenotypic changes in morphologically normal TSC- and VHL-mutant renal epithelial cells: The AKT-TSC pathway regulates ribosomal protein gene expression

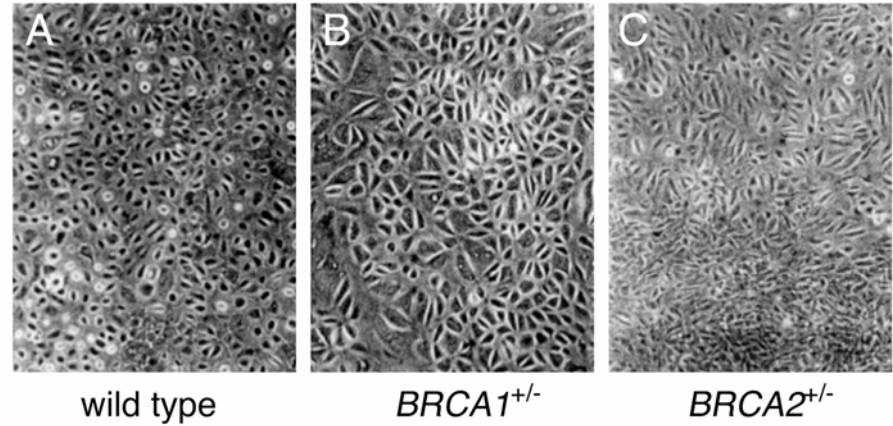


BRCA1/BRCA2-associated cancers: lifetime risk

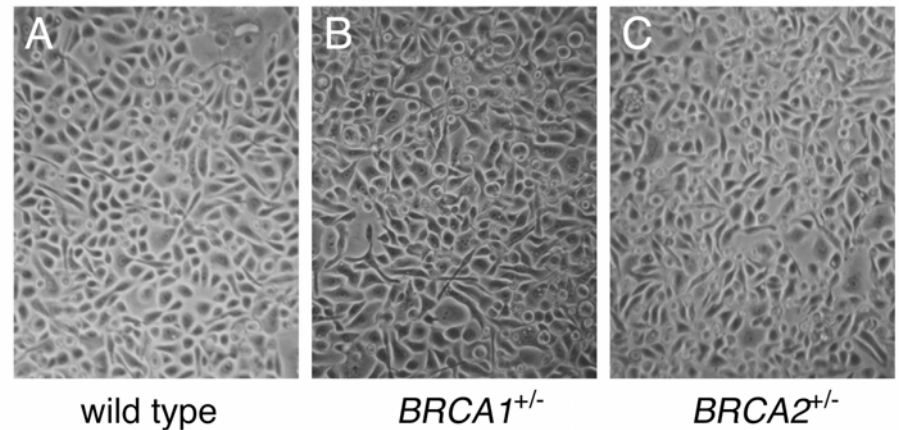


Samples from healthy subjects and subjects carrying BRCA1 and BRCA2 mutations were analyzed by microarray analysis, processed by Robust Multi-chip Average method and validated by Real Time PCR

Human ovarian surface epithelial cells



Human breast epithelial cells in culture



Breast and Ovarian Cancer

- BRCA1 and BRCA2 mutations were associated with differential gene expression

Cell Cycle and Growth Control	Cdc2, cyclin B
Cell-cell and cell-matrix adhesion	Tensin 4, mucin 16, keratin 14
Signal Transduction	RGS4, regulator of G protein signaling

- BRCA1 mutations are associated with cell-mobility and locomotion genes in breast cancer, and with cell cycle regulation genes in ovarian cancer.
- Alterations in the gene expression pattern associated with single-hit mutations of BRCA1 and BRCA2 tumor suppressor genes are likely to represent the earliest molecular changes in the development of breast and ovarian cancer.

Intellectual Property

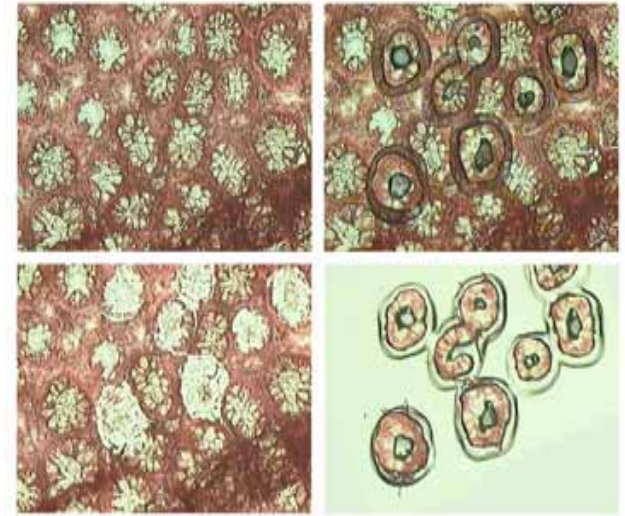
- PCT/US06/47222 - Filed December 11, 2006
- Title: “Methods and Compositions for Assessing Alterations in Gene Expression Patterns in Clinically Normal Tissues Obtained from Heterozygous Carriers of Mutant Genes Associated with Cancer and Methods of Use Thereof”

This discovery applies to any gene mutation that creates a dominantly inherited predisposition to cancer: tumor suppressor genes, oncogenes and DNA repair genes

	BRCA1	BRCA2
Breast	Mammaglobin Lipophilin B Tensin 4 Mucin 16	Tensin 4 Mucin 16 Keratin 14
Ovary	Cyclin B1 Cdc2 NUSAP-1 CENP-A CD26 SAA2 Ponsin CHI3L1	CHI3L1 MMO3 COX-1

Colorectal Cancer - LCM

Microarray Analysis of RNA from Target Epithelia
Processed by Laser Capture Microdissection



LCM of colonic epithelium
from control & predisposed
individuals (FAP patients)

RNA extraction &
amplification

Hybridization to
Affymetrix arrays

Normalization &
data analysis

Identification of
candidate
biomarkers

Biomarker
validation



Value Proposition

Differentially expressed genes may lead to:





- **Diagnostic applications**

Laboratory tests to diagnose heterozygosity for mutant tumor growth suppressor genes and to establish a reliable screening to stratify the population into classes of cancer risk.

- **Therapeutic applications**

Molecular targets for strategies of intervention based on novel chemopreventive agents.

Technology Summary

- Hereditary cancer syndromes provide a unique opportunity to develop and test novel prevention strategies 
- Heterozygosity for tumor suppressor gene mutations is associated with detectable changes in expression profile representing early molecular changes in tumorigenesis 
- These mRNAs represent a genetic signature of cancer. This discovery may be also applied to protein changes detectable with a proteomic approach 
- Early molecular alterations may include both early markers of transformation and genetic changes driving transformation (*targets for chemoprevention*) (microarray-based approach in kidney cells allowed identification of “druggable” targets) 

What is Next?

To collect additional data and perform validation studies.

To confirm expression of protein(s) from mRNAs that are up or down-regulated in subjects carrying BRCA1 or BRCA2-mutated genes

To associate the altered expression of mRNAs and proteins to an increased risk of cancer.