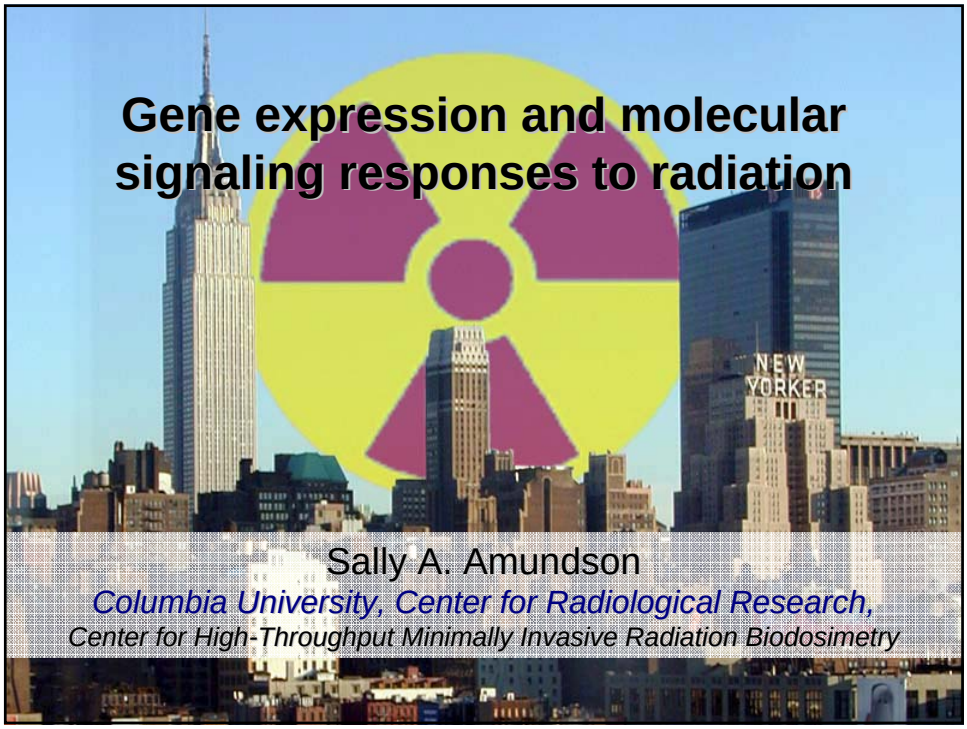

Gene expression and molecular signaling responses to radiation

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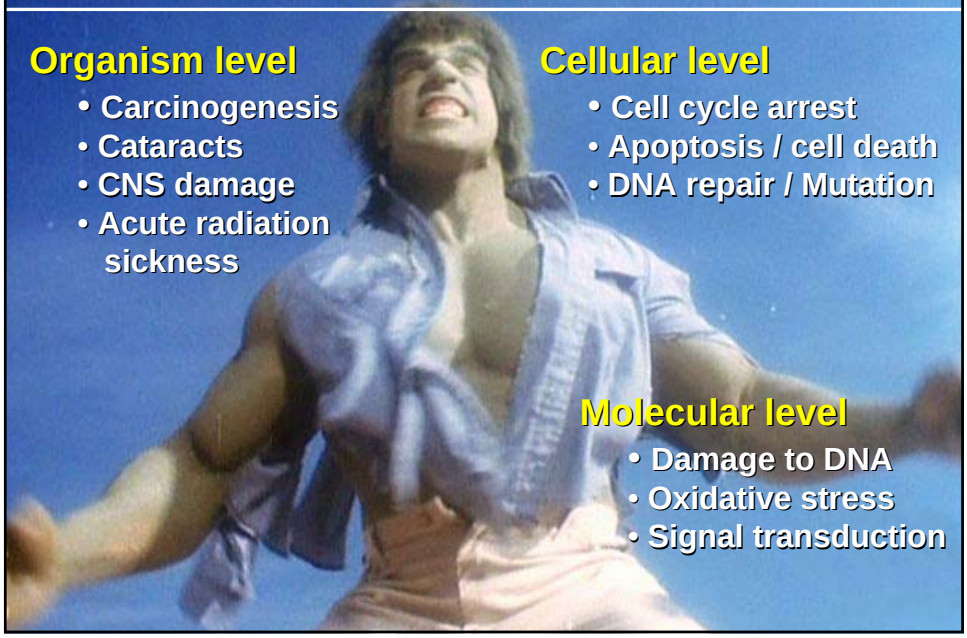
Center for High-Throughput Minimally Invasive Radiation Biodosimetry

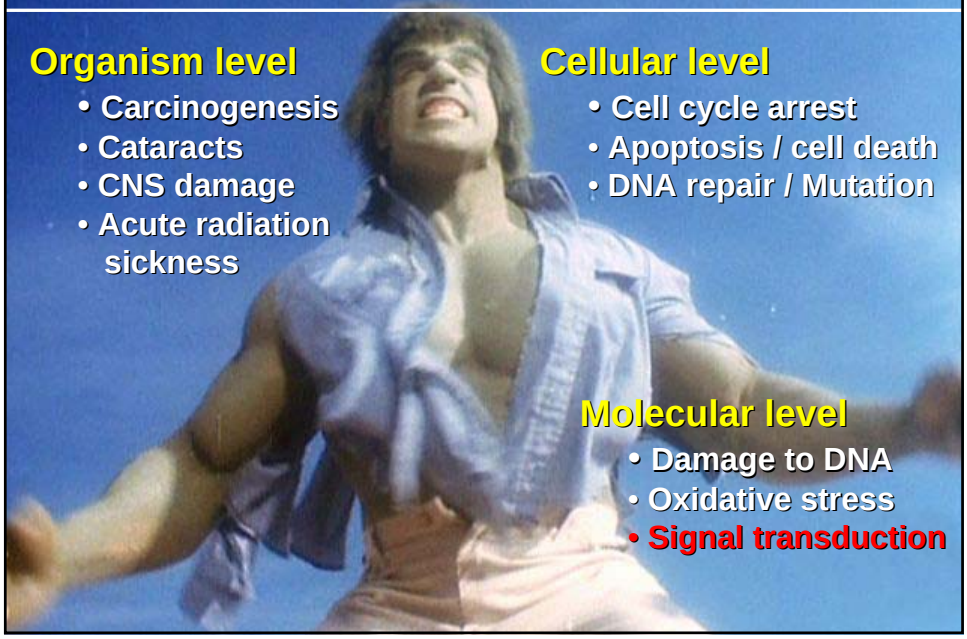
General Overview

- Many signaling pathways respond to ionizing radiation
- Radiation causes gene expression changes
- Gene expression changes underlie many cellular responses
- We can now study these changes in gene expression across the whole genome
- Gene expression profiles may soon provide useful information for biodosimetry

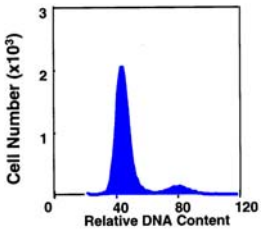
Ionizing radiation effects on living cells



Ionizing radiation effects on living cells



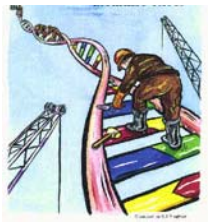
Cellular consequences of radiation exposure

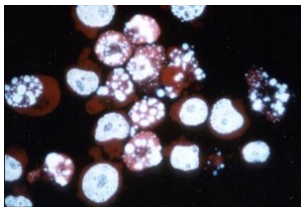


Cell cycle arrest (cells stop dividing)

- Temporary or permanent

DNA repair

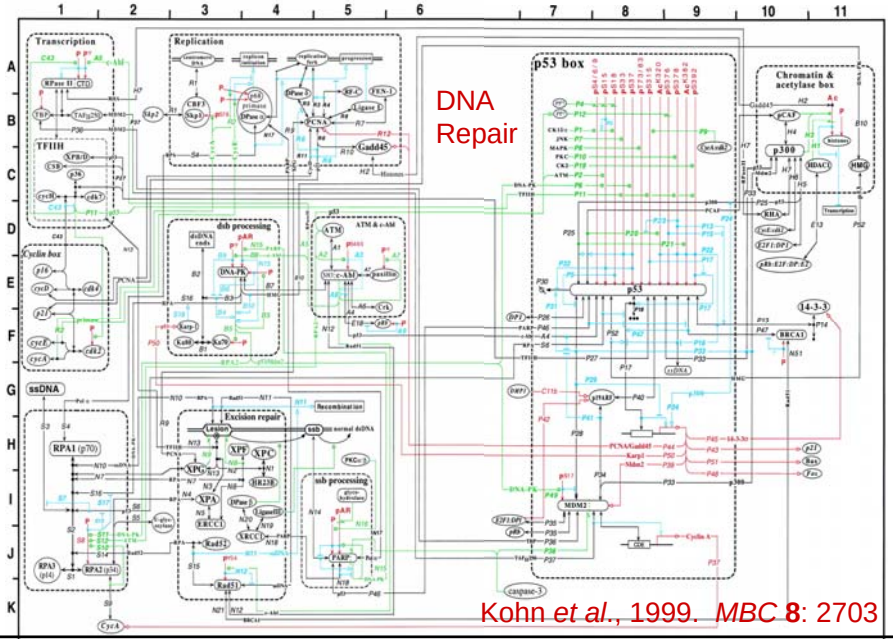




Cell Death

- Apoptosis or necrosis

Complexity of cellular signal transduction



Kohn *et al.*, 1999. *MBC* **8**: 2703

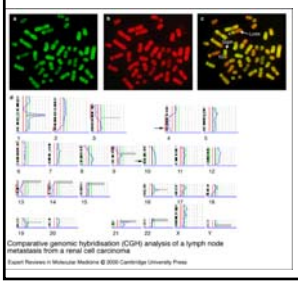
The “omic” revolution in biology

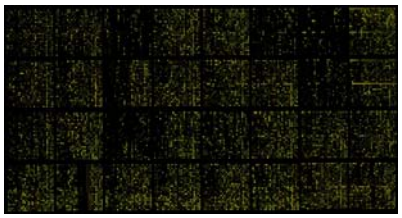
“-ome”: “an abstract entity, group or mass.”

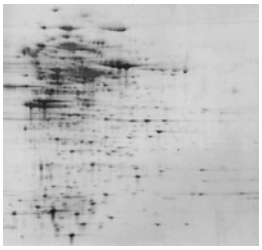
~Webster’s Third New International Dictionary

Hence, **“-omics”:** the study of groups of things

- DNA - genome
- RNA - transcriptome (“functional genomics”)
- Protein - proteome







Profiling radiation responses in diverse cell lines

The NCI60 cell lines

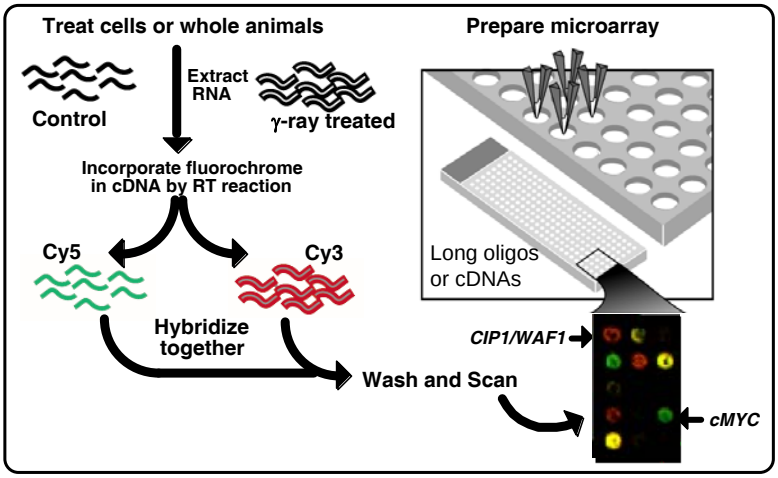
NCI60 Cell Panel
- 60 cell lines derived from different types of cancers
- Well-characterized

Survival following radiation exposure

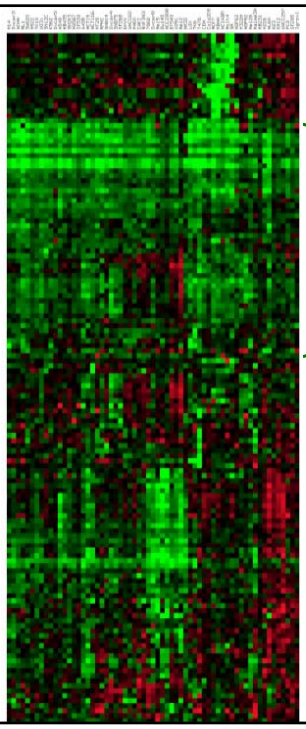
Microarray Hybridization
- Functional genomics approach to measure global gene expression

Compare this data to radiation survival

Functional Genomics: Expression microarrays

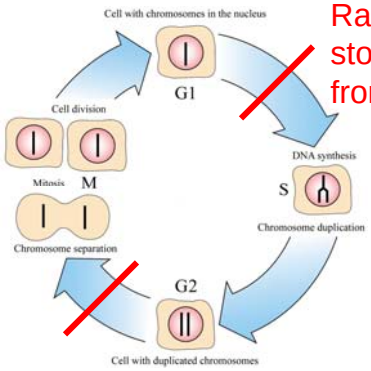


Provides a "snapshot" in time of the relative expression levels of essentially the entire genome. Compare tumor to normal, response to stress (e.g. irradiation) etc.



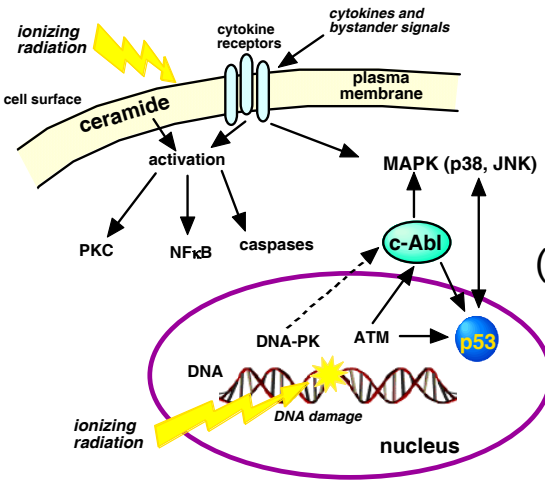
"Universal" down-regulated Radiation response cluster

Many genes that regulate cell cycle progression



Radiation stops cells from dividing

Stress Signaling Pathways



Protein modifications

↓

Gene expression changes (Functional Genomics)

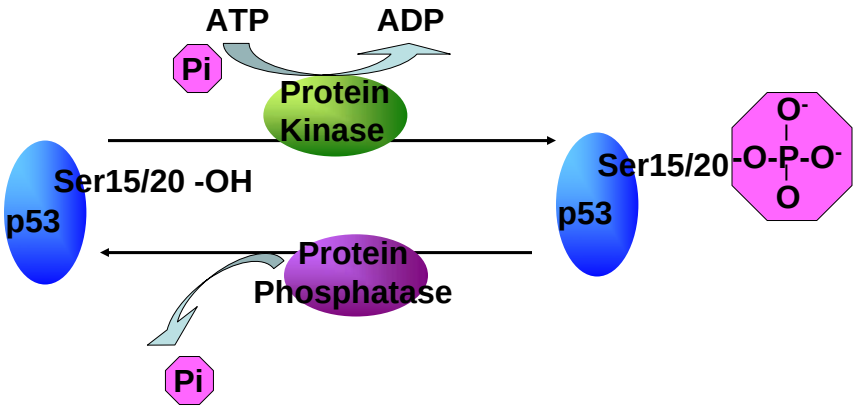
↓

Altered cellular function

Phosphorylation of p53 in response to radiation

Radiation causes phosphorylation of p53 protein at several sites

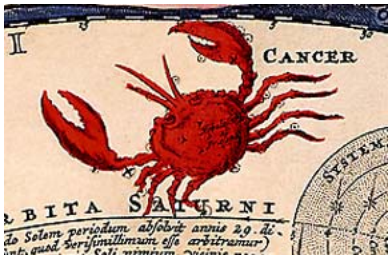
- stabilizes p53 and makes it accumulate.
- modifies p53's function as a transcription factor



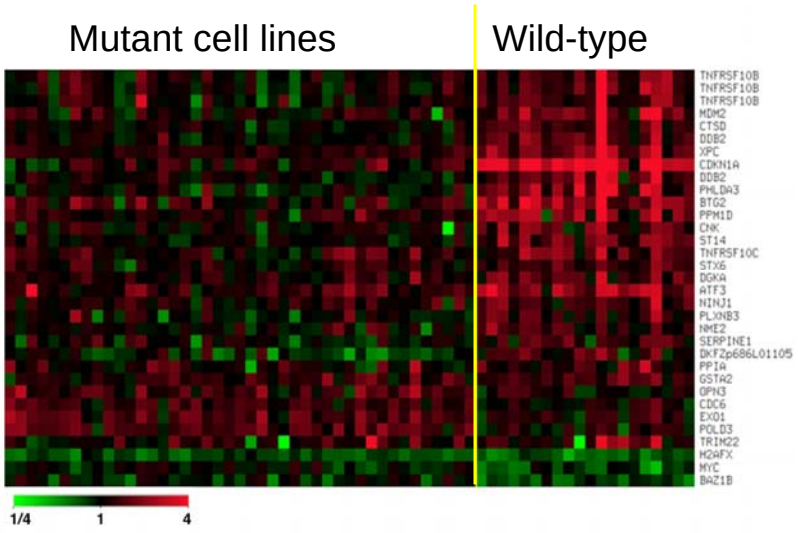
p53 status in the NCI60 cell lines

p53 is the most frequently mutated oncogene in cancers

72% of the NCI60 cell lines have mutant p53

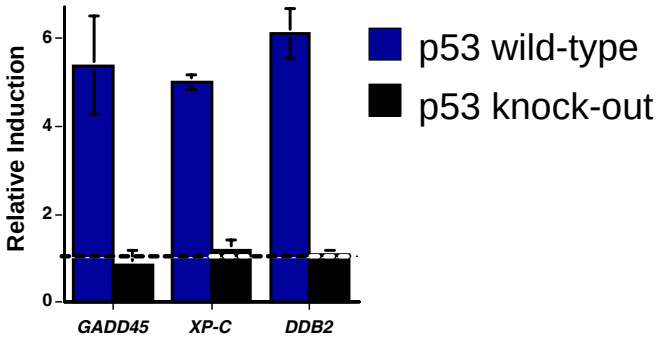


p53-regulated gene cluster

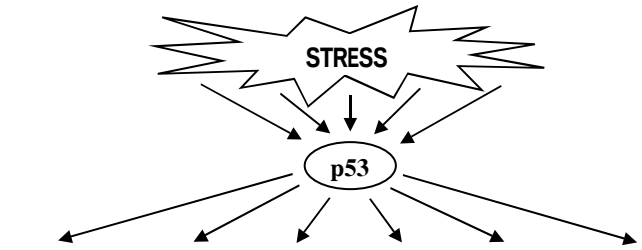


Some genes require p53 for radiation response

Human cell lines irradiated with γ-rays



Some common p53 effector genes



Cell Cycle	Apoptosis	DNA Repair	Senescence	Anti-angiogenesis	Other
CIP1/WAF1	*BAX*	*XPC*	*CIP1/WAF1*	*VEGF*	*MDM2*
ClnG	*BCL-X*	*DDB2*	type I PACAP	*IL-8*	*FRA1*
ClnD1	*PAG608*	*GADD45A*		*HIF1α*	*ATF3*
WIP1	*FAS/APO1*	*PCNA*		*MMP2*	*14-3-3σ*
EGF-R	*KILLER/DR5*	*p53R2*		*BAI-1*	*Rb*
Rb	*TRUNDD*			*TSP1 & 2*	*c-MYC*
BTG2	*TRID*				*ninjurin*
PCNA	*SIAH*				*MAP4*
GADD45A	*IGF-BP3*				*amyloid*
14-3-3σ	*PIG1 to PIG14*				*PIR121*
TGF−α	*14-3-3σ*				*WIG1*
SIAH	*MAP4*				
IGF-β	*TSP1 & 2*				
PIG1 to PIG14	*bFGF*				
inhibin-β	*GML*				
GML					

Determining the outcome of p53 activation

A complex interplay of factors determines the outcome of p53 activation in cells. These can include:

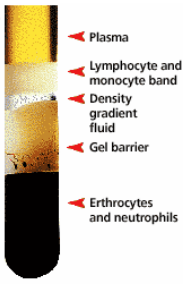
- Dose: less DNA damage at lower doses is associated with cell cycle arrest, more damage and higher doses with apoptosis.
- Cell type (lymphoid cells more likely to undergo apoptosis, fibroblasts more likely to undergo terminal arrest or necrosis.)
- Extracellular stimuli - signals from the environment (growth factors, cytokines etc.)
- Specific modifications of p53 alter its activity differently.
- Binding to interacting proteins (p300, CBP, MDM2, Rb, c-Abl, p73 etc.), competition with other proteins for cofactors.
- Differential activation of effector genes.

Gene expression for radiation biodosimetry and triage

Why biodosimetry?

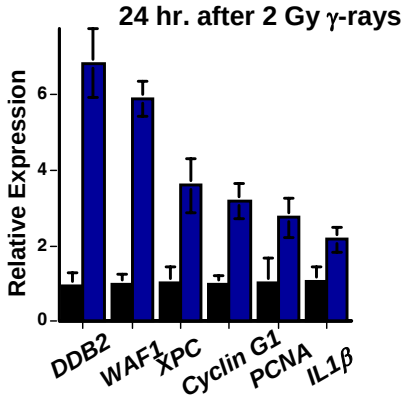
Irradiation of human peripheral blood lymphocytes

- Separate lymphocytes from whole blood
- Split culture: half + 2 Gy γ-rays, half control
- Harvest RNA after 24 hours, compare on microarray
- Follow-up of induced genes
  - Reproducibility in multiple donors
  - Time-course of response
  - Dose-response relationship



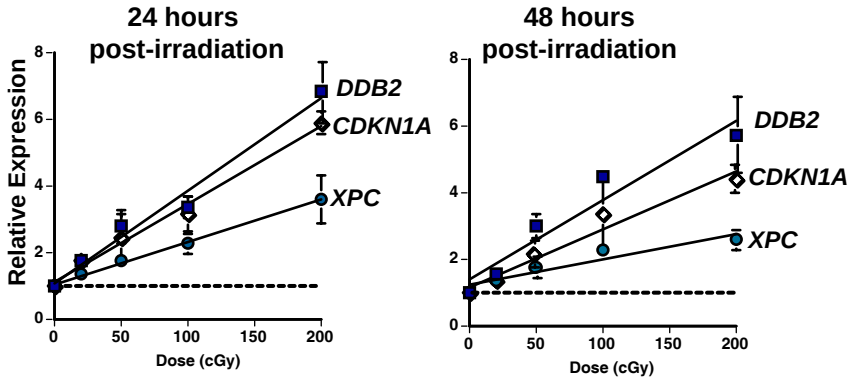
Identification of potential radiation biomarkers

Markers up-regulated on microarray of γ-irradiated PBLs



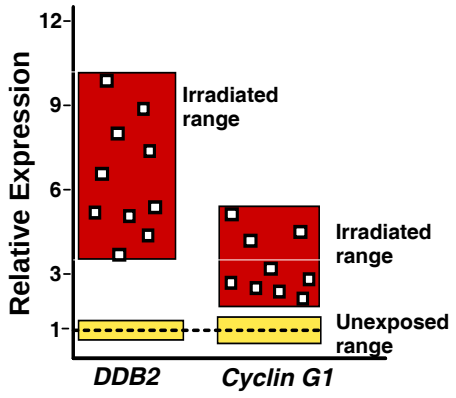
Amundson *et al.*, (2000)
Radiation Research, 154 (3): 342-346

Gene induction in PBL persists at least two days



Amundson *et al*., (2000)
Radiation Research, 154 (3): 342-346

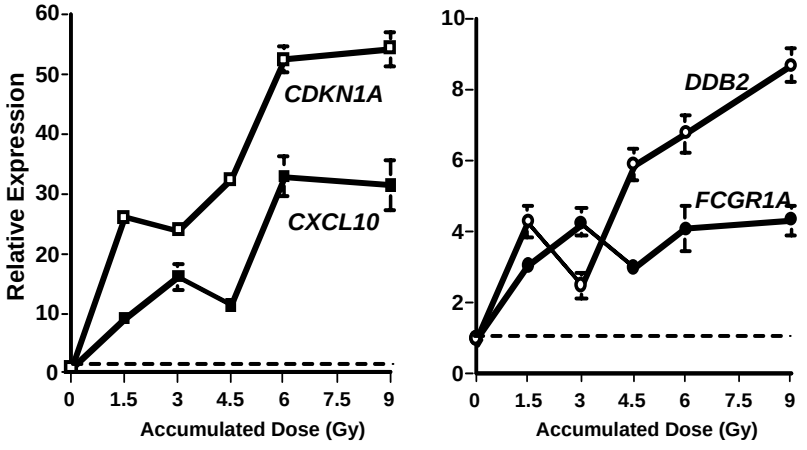
Expression ranges to estimate exposure without knowledge of pre-exposure levels



Human in vivo total body irradiation

- Patients for stem cell transplantation
  - collaboration with Joel Greenberger lab (U. Pittsburg)
- 2 x 1.5 Gy fractions per day for 4 days
- Blood drawn before treatment, 6h after first fraction
- RNA extracted and hybridized to microarray
- Follow-up of induced genes by Real-Time PCR
  - Time-course and dose-response
  - Response in multiple donors

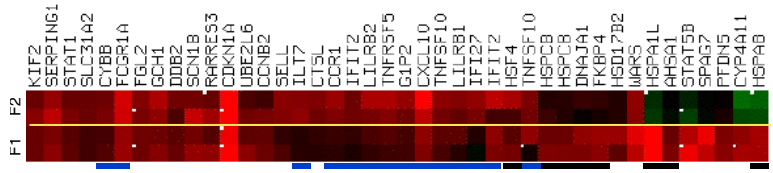
Gene induction through a course of TBI



Amundson *et al.*, (2004)
Cancer Research, 64: 6368-6371

Array analysis of *in vivo* irradiation

First 2 TBI fractions



Functional Category	EASE Statistic	Fisher Exact
Heat Shock	0.00973	0.000443
Immune Response	**6.8×10^{-12}**	**1.31×10^{-12}**

Amundson *et al*., (2004) *Cancer Research*, **64**: 6368

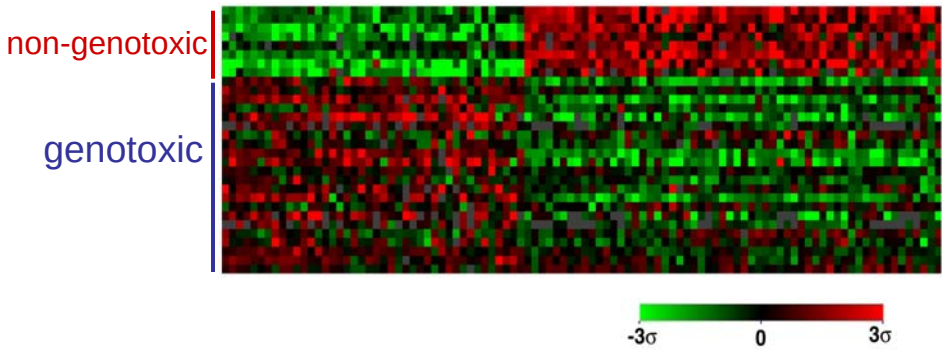
Specificity of radiation signatures

- Discrimination from non-radiation stress
  - Inflammation / infection
  - Trauma (*e.g.* burn injury)
  - Drug treatments (*e.g.* chemotherapy)
- Examination of potential confounding variables
  - age
  - gender
  - smoking status

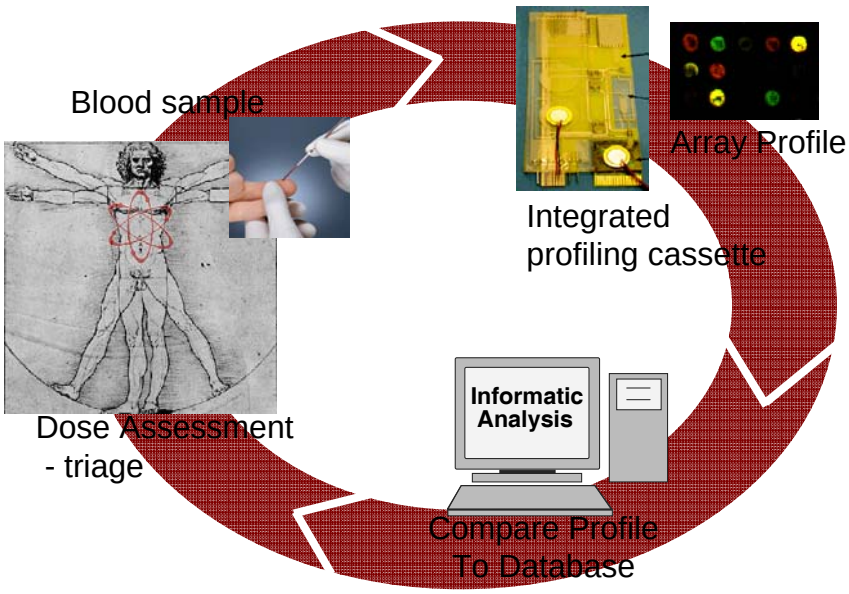
Genes discriminating genotoxic from non-genotoxic stresses

Two cell lines treated with 12 different stress agents representing different modes of action, including:

- Genotoxic agents (DNA-damaging)
- Non-genotoxic agents (don't damage DNA directly)



Developing gene expression for biodosimetry



Summary

- Many complex signaling pathways are activated by radiation.
- Activation or change in specificity of transcription factors and the expression of effector genes is a common means to modulate the outcome of radiation exposure.
- These complex systems allow cells to “fine-tune” responses and produce different outcomes in different tissues or environments.
- Gene expression changes also occur *in vivo*, and could form the basis for biodosimetry and triage.

Supported by the *Center for High-Throughput Minimally Invasive Radiation Biodosimetry* through NIAID grant #U19 AI67773-01