2-D and 3-D Esophageal Epithelial Cell Systems for Radiation Risk Assessment

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October 22, 2009
Overview

• Space Radiation and Human Health Risks
• Focus on Esophageal Cancer
• Biological Models
• DNA Damage and Repair
• Future Work
Crew Health Risks in Space

- **Microgravity**
  - Bone resorption
  - Muscle atrophy
  - Reduced immune system function
  - Cardiovascular and neurovestibular adaptation

- **Nutritional Deficiencies**

- **Increased Radiation Exposure**
Space Radiation

Sources:

- Trapped radiation
- Solar particle events - protons
- Galactic cosmic rays* - protons + HZEs

→ High energy particles cause unique damage to biomolecules, cells, and tissues

→ No human data to estimate risk from heavy ion damage

http://spaceflight1.nasa.gov/shuttle/support/researching/radiation/brochure1/dnahelixlg.jpg
Space Radiation Risks

Carcinogenesis:
- Leukemias
- Solid cancers

Degenerative Tissue Effects:
- Heart disease
- Cataracts
- Respiratory diseases
- Digestive diseases

Central Nervous System Damage:
- Motor skills
- Behaviors
- Accelerated aging

Acute Risks:
- Death
- Vomiting/nausea
Why Esophageal Cancer?

- High excess relative risk in A-bomb survivors
- Dietary impacts
  - Nutritional deficiencies
  - Dietary carcinogens

→ Because stomach and esophageal cancers are important components of total cancer risk, understanding radiation carcinogenesis and potential role of nutrition are open questions that need to be addressed.
Physiology

Types of Epithelium

- Simple squamous
- Simple cuboidal
- Simple columnar
- Transitional
- Stratified squamous
- Stratified cuboidal
- Pseudostratified columnar

http://www.bio.psu.edu/courses/fall2003/biol129/powerpoint/Ch05/sld004.htm
Physiology

H&E staining of normal esophageal epithelium

differentiated zone
epibasal layers
papillary basal layer
interpapillary basal layer

Seery et al., J Cell Science 2002
Pathology

Two major types:

Esophageal Squamous Cell Carcinoma (ESCC)

Esophageal Adenocarcinoma (EAC)

www.oncologychannel.com
Pathology

Barret’s esophagus → Adenocarcinoma → Squamous cell carcinoma

→ High mortality associated with these carcinomas

http://www.pathology.med.ohio-state.edu
Epidemiology

• 7th leading cause of cancer death in the U.S. and the 6th leading cause of death worldwide

• More common in men than women

• ACS estimates that approximately 16,470 new esophageal cancer cases will be diagnosed and 14,280 deaths from esophageal cancer will occur in the United States

• Foci of high incidence ESCC exist in the “esophageal cancer belt”, encompassing an area stretching from northern Iran eastward through Central Asia and into northern China. Also region of high-incidence areas in South America, which includes northeastern Argentina, southern Brazil, Paraguay, and Uruguay.

• The incidence of ESCC in these geographical clusters 10 to 100 times higher than in the United States.
Risk Factors

ESCC:
- Geographic location
- Tobacco and alcohol
- Untreated achalasia
- History of caustic injury and drinking hot beverages
- Dietary factors (nitrosamine intake from preserved food, selenium, β-carotene, zinc, vitamin A and vitamin E deficiency, etc.) and general malnutrition
- Particulate inhalation
- Human papilloma virus infection (HPV-16 and -18)
- **Ionizing Radiation Exposure**

EAC: Reflux – Barrett’s Esophagus, Obesity
Multistage Progression

Hyperplasia → Dysplasia → Carcinoma in situ → Metastatic Cancer

Telomerase, p53, p16
Cyclin D1, EGFR

→ **Stepwise accumulations** of multiple genetic alterations lead to the activation of oncogenes and/or the inactivation of tumor suppressor genes

→ The differential expression of these critical genes and their downstream effectors enables cells to **override machinery of normal growth control**
Cell Types

- Normal esophageal epithelial cells (HEECs)
- Immortalized cell line (EPC-hTERT)
- Genetically Modified Cell Lines:
  - p53 (R175H – loss of function mutation)
  - EGFR (upregulated expression)
  - p53-EGFR (double mutant)

Represent “snapshots” in stages of esophageal cancer progression from normal to malignant

Cell Lines generously provided by Dr. Anil Rustgi, University of Pennsylvania
Biological Models

- 2D Culture
- 3D Culture
- Animals
2D Models

→ Culture esophageal epithelial cells in vitro
  - preferred over explants (labor-intensive, standardizing difficulties)

**static culture flasks**
- Serum-free media
- Hormone supplementation

**feeder layers**
- Serum-supplemented media
- Irradiated fibroblast feeders
(◊) EPC2; (●) EPC2-hTERT

Arrow indicates time of transduction

→ EPC2-hTERT cells overcame senescence and continuously grew, without a slow growth phase
Why 3D?

• More closely mimic in vivo mechanical and microenvironmental conditions
  • Morphological features
  • Differentiation markers
  • Growth characteristics

• Can study interaction between tissue compartments

→ Better model for risk assessment
3D Models

Spheroids in Matrigel

Organotypic Cultures with Air-Lift Interface (ALI)
Spheroids in Matrigel

→ Use the “overlay method” with Matrigel to generate spheroids

Debnath et al., *Nature Reviews Cancer* 2005
3D Organotypic Culture

- Stratified layers of epithelial cells
- Collagen/ECM matrix with fibroblasts
- Acellular layer of collagen
- Medium
3D Organotypic Culture

→ Use collagen/Matrigel/fibroblast base, air-liquid interface

Dongari-Bagtzoglou et al., Nature Protocols 2006
Acknowledgements

• Francis Cucinotta – NASA JSC
• Megumi Hada – USRA
• Anil Rustgi and lab members, U Penn
• Colleagues at NSRL and NASA-JSC
• Work supported by funding from DOE and NASA
Questions?