Molecular Mechanisms of Esophageal Cancer

Anil K. Rustgi MD
University of Pennsylvania
GI cancer models and translational medicine

- Opportunities for chemoprevention of preneoplastic lesions
- Development of new molecular diagnostics – proteomic approaches
- Development of molecular targeted therapies as an adjunct to conventional therapy or supplant such modalities.
BACKGROUND

Epidemiology
- Esophageal squamous cell cancer (ESCC) is common worldwide and amongst top 10 cancers in US
- Esophageal adenocarcinoma (EA) is also among top 10 cancers in US

Etiology
- ESCC: environmental factors (cigarettes/alcohol), genetic alterations (EGFR, cyclin D1, p16, p53)
- EA: environmental factors (acid reflux), Barrett’s esophagus (intestinal metaplasia of normal squamous epithelium)

Clinical
- Late onset presentation of symptoms associated with advanced stage of disease, and thus, poor prognosis
- Management combines surgery, radiation and chemotherapy.
- Thus, there is need for better diagnostic markers and therapeutic approaches.
STRATIFIED SQUAMOUS EPITHELIUM OF THE ESOPHAGUS

- **Apoptosis**
- **Differentiation**
- **Proliferation**

**Layers**
- **Suprabasal layer**: Involucrin, TG, K4/K13, ETS-1
- **Supraborasal layer**: K5/K14, EGFR, KLF4, KLF5
- **Basal layer**: K5/K14, EGFR, KLF4, KLF5
- **Stroma**

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**Suprabasal layer**
- Involucrin
- TG
- K4/K13
- ETS-1

**Supraborasal layer**
- K5/K14
- EGFR
- KLF4
- KLF5

**Basal layer**

**Stroma**

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**Apoptosis**

**Differentiation**

**Proliferation**
Projects

- Isolation and characterization of stem cells
- Molecular regulation of proliferation-differentiation gradient
- Modeling carcinogenesis: interplay of oncogenes and tumor suppressor genes
Keratinocyte stem cells are associated with ECM and give rise to TA cells

Stem Cell
- Cycle slowly
- Self Renewing
- Resist apoptosis
- Maintain telomere length
- Give rise to progenitor cells
- Represent a minor population

Integrins $(\alpha 6, \beta 1)$

ECM (Fibronectin, Collagens, Laminin, etc.)

Differentiating Cells

Transit Amplifying Cell (Daughter)
Mouse Tissues

BrdU (0.8mg/ml) for 4 weeks
Sacrificed at time point 0
Mouse Tissues

BrdU (0.8mg/ml) for 4 weeks
Sacrificed at time point 4 weeks
BrdU label retaining cells localize throughout the esophagus

- **Upper esophagus**: 0.1-1%
- **Mid-esophagus**: 0.1-1%
- **Distal esophagus**: 1-3%

bronchus

diaphragm
SP cells were detected within esophageal epithelial cells with Hoechst 33342 dye, which were abolished by Verapamil, an inhibitor of Hoechst 33342 dye efflux.
Gene array to identify stem cell markers

FACS/sorting of esophageal epithelial cells

SP cells

Non-SP cells

mRNA Amplification
2-rounds of \textit{in vitro} transcription
(Affimmetrix GeneChip probe array)

>5X upregulation in SP
(629 genes)
  ABCG2 (responsible gene for SP)
  CD34
  Cell adhesion
  Extra cellular matrix (ECM)
  Cell-cell communication

>5X downregulation in SP
(492 genes)
  \(\alpha_6\)-integrin
  Proliferation
  Cyclins
  Transcription factors
Colocalization of BrdU (LRC) and CD34

Red: DAPI   Blue: CD34   Green: BrdU
Functional assays of stem cells: principle is to demonstrate self-renewal

1. Colony formation assay

2. *In vivo* transplantation (epithelial reconstitution) assay
Colony formation assay with SP and CD34+ cells
Projects

- Isolation and characterization of stem cells

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- Modeling carcinogenesis: interplay of oncogenes and tumor suppressor genes
Modeling cancer in esophageal squamous epithelial cells, using in a three-dimensional cell culture system: complementary approach to mouse models

1. Organotypic culture

2. Retrovirus vectors
   (transduced in human esophageal cells (EPC))
   - EGFR
   - Tamoxifen inducible constitutively active form of AKT
   - hTERT
   - Cyclin D1
   - Mutant p53
EPC-EGFR/hTERT cells in 3D organotypic culture

40x

100x

200x

400x
EPC-EGFR/hTERT/mutant p53 in 3D organotypic culture: invasive malignant cells with identical phenotype observed in human esophageal squamous cell cancers.
Combination of EGFR-hTERT-mutant p53 with invasive tumor cells
EBV ED-L2 Promoter Targets Cyclin D1 to the Oral-Esophageal Squamous Epithelia in Transgenic Mice
Oral-esophageal cancer in L2D1+/p53+-mice
Lymph node: H & E

Lymph Node: keratin IHC
Models of cancer: intervention

Normal → Dysplasia → Cancer → Metastasis

- Prevention/chemoprevention
- Diagnosis
- Therapy
Sulindac reduces dysplasia in L2D1+/p53+-/+ mice

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Genetic Pathways in Esophageal Squamous Cell Carcinogenesis

Normal
- Regulated proliferation
- Differentiation
- Death

Cancer
- Autonomous Indefinite
- Cell proliferation
- (immortal/transformed)
- Metastasis

Regulated proliferation
Differentiation
Death

EGFR

ras

Cyclin D1
p16^{INK4A}

Telomerase

Zn^{+2} deficiency
NMBA

p53

p14^{ARF}

p53

p14^{ARF}
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