HUMAN GENOME

30,000 genes.

Length of DNA/chromosome
Six feet

23 chromosomes
Molecular Aspects of Carcinogenesis
Human Papilloma Viruses
Precancerous Oral Lesions
Papillary Lesions

Oral Kaposi's sarcoma associated with acquired immunodeficiency syndrome among homosexual males.

Abstract

Clinical disease states encountered in the acquired immunodeficiency syndrome (AIDS) have been reviewed with an emphasis on oral Kaposi's sarcoma. The disease is reaching epidemic proportions among homosexual males and is characterized by onset of fever, malaise, diarrhea, and lymphadenopathy. Subsequent to these initial nonspecific signs and symptoms, patients develop a variety of opportunistic infections or Kaposi's sarcoma (or both). The oral lesions of Kaposi's sarcoma are characterized by red, blue, or purple plaques or nodules encountered primarily, yet not exclusively on the palate. Other oral manifestations of AIDS include candidiasis and herpetic stomatitis. Epidemiologic studies suggest the probability of a transmissible agent, perhaps a virus. It is recommended that dental care should be rendered to these patients, using mask and gloves with autoclave sterilization of all instruments.
Cancer Biology
SMOKING GUNS AND FOOTPRINTS

MOLECULAR EVIDENCE
CANCER IS A LESION OF THE GENOME

PROLIFERATION
REPLICATION
REPLICATION
ADHESION
VASCULAR PENETRATION
INVASION
ACCELERATION
braking systems
CONSTITUENT PARTS
MITOSIS
THE CELL CYCLE

• GROWTH FACTORS, ONCOGENES
• GROWTH FACTOR RECEPTORS
• INTERNAL SIGNALING PATHWAYS
  – PROTEINS AND ENZYMES
CIRCADIAN RHYTHMS

- Loss of leaves in fall, renewed in spring
- Menstrual cycle
- Tooth eruption
- Cellular clocks
Abrogation of ProMitotic Transcription
Defective Tumor Suppressor Proteins

Promotor Methylation
- Transcription Factor
- CH3
- No Transcription

Deletion
- Transcription Factor
- No Transcription

M P
- Nonfunctional Proteins

Homozygous Wild Type (Normal)
Heterozygous 1 Wild Type 1 Mutated
Homozygous Both Mutated

Loss of Heterozygosity
Tumor Suppressor Proteins in Head and Neck Carcinoma

- LOH or microsatellite instability on various chromosomes and numerous loci: 86%
  - LOH TRAIL Receptor DR4(8p21-22): 47%
  - Methylation or LOH CDKi p16(9p21): 80% early
  - LOH p53(17p13): 50% during invasive stages
  - LOH PTEN: 10%
  - pRb: >10%
Oral Keratinocyte Carcinogenesis

- The Path to a Malignancy
  - Increased cell cycling
  - Increased keratinocyte motility
  - Keratinocyte-ECM interactions and invasion
  - Tumor cell adhesins that tether to vascular basal lamina proteins
  - Vascular penetration and metastasis

- The Molecular Basis is Multifactoral and Multiphasic with Activation of some and suppression of other genes
  - Mitosis activation
  - Cytoskeletal changes
  - Adhesion molecule expression
Protooncogene Upregulation

- Cyclin D1: 30%
- P63 signaling/migration protein: 30%
- Epidermal GFR: 10%
- C-Myc
- Jun
- RAS
Sequential Molecular Stages in Oral Carcinogenesis

NORMAL
Extant Heterozygous Germ Line Mutations
LOH

HYPERPLASIA
HPV
9p12 p16

DYSPLASIA/OIN
HPV
17p13 p53
3p21, 11q13, 13q21, 14q32, 10q23

SQUAMOUS CA
8p21-22 TRAILRec
Cyclin D1 RAS
EGFR MYC JUN

modified from Forastiere, Koch, Trotti and Sidransky: NEJM 345:1890, 2001
Biomarkers

- Biologically expressed phenomena that when detected in a tissue, predict an outcome behavior.
- In the conventional sense, a biomarker is demonstrable microscopically by special techniques or may be detectable by molecular or biochemical methods.
- In reality, even clinical signs may be considered biomarkers.
Histopathological Biomarkers

• Expression of Protein, DNA or RNA sequences

• Proteins
  – Immunohistochemistry

• DNA
  – In-situ Hybridization, PCR

• RNA
  – In-situ Hybridization, RT PCR
Biomarker Classes

• Proliferation Markers
  – Cell Cycle Proteins, Oncoproteins, Mutated Tumor Supressors

• Invasion Markers
  – Basal lamina proteins, extracellular matrix, ECM enzymes

• Nuclear Qualitative Markers
  – Computerized Image analyses

• Nuclear Quantitative Markers
  – Ploidy

• Viral Markers
  – HPV 16
The Ideal Biomarker

- A procedure that can be performed on biopsy specimens
- Expression is easily demonstrable
- Expression of the marker is predictive for cancer progression risk in dysplastic oral lesions
- Expression of the marker is predictive for cancer progression risk in nondysplastic oral lesions
- False negative and positive results do not occur (i.e. expression is 100% predictive for cancer progression, lack of expression is 100% predictive for no cancer progression risk)
THE CARDINAL RULE OF ORAL EPITHELIUM: ONLY THE BASILAR AND PARABASILAR CELLS DIVIDE
Expression of promitotic genes or their protein products in the spinous cell layer is indicative of intraepithelial neoplasia.
BENIGN KERATOSIS

DYSPLASIA

P53
Benign Keratosis

Dysplasia

PCNA
HUMAN PAPILLOMA VIRUS
HPV
Uterine Cervix
Oncogenic HPVs and Oral SSC

- Oncogenic E6, E7 immortalize oral keratinocytes; Hyperplasmiagenic E6, E7 do not.
- Oncogenic types are integrated into host DNA and are truncated with loss of the E1, E2 transcription control elements.
- HPV is sufficient for Immortalization yet is not sufficient for Tumorigenesis when transplanted into immunodeficient mice.
- HPV requires other genetic lesions as cofactors for carcinogenesis.
- Infected cells have been shown to express over 80 genes that are not ordinarily expressed in noninfected cells.
- The molecular pathogenesis has been shown to be similar to that of CIN and cervical carcinomas.
COTTONTAIL RABBIT papillomavirus

• Papillomas cottontail rabbit

• Human Papilloma Viruses
  – HPV 2, 4 skin and plantar Warts
  – HPV 6, 11 condyloma acuminatum, laryngeal
  – HPV 16, 18 and others carcinoma
    • uterine cervix, vulva, anus, penis, oropharynx, oral cavity
HPV16

ABROGATION OF APOPTOTIC PATHWAYS
LOSS OF DNA REPAIR FUNCTIONS
DNA DAMAGE PRONE
ACTIVATION OF CELL CYCLE

UBIQUITIN DEGRADATION

LOSS OF G1/S CHECKPOINT

E2F

HPV 16 E6 Oncoprotein

p53

Cyclin D1

CDK4

CDK6

HPV 16 E7 OP

pRb

pRb

E2F

P

CDK2

Cyclin A

Cyclin E

G1/S transition

ProMitotic Transcription
HPV16 E7

Mitogenic
- Cyclin D1
- CDK4
- CDK6

Cycle Arrest
- p130
- E2F
- p107
- E2F
- pRb
- E2F
- E7
- p130
- p107
- pRb

G1/S transition
- CDK2
- Cyclin A
- CDK2
- Cyclin E

Phosphorylation of POCKET PROTEINS
- P
- E7
- p130
- P
- E7
- p107
- P
- E7
- pRb
- P

ProMitotic Transcription
- E2F
P53 Degradation

- Loss of p53 apoptotic pathway
- G1/S checkpoint over-ride
- Abrogation of DNA repair
- Somatic mutation frequency increased
E6-AP1 shares homology with GAP

GAP ACTIVITY

Rap1, Rap2

GTP

GDP

E1 activating enzyme

E2 transfer enzyme

E6

E6-AP1

p53

Ubiquitin

Ubiquitin

ATP

26s Proteasome

DEGRADATION OF E6-AP1 GAP ACTIVITY

GTP Loading of Rap

MITOGENIC SIGNALING CYTOSKELETAL CHANGES
HPV E6 has pleiotropic effects!

- Transfection causes immortalization but is not tumorigenic
- E6 binds to p53>ubiquitination
- Binds to Host protein E6-AP, a facilitator for p53 binding; occurs only for oncogenic HPV E6
- Binds to Host protein E6-AP, degradation, activation of Rap1
- Telomere maintenance, activation of telomerase
- Binds to hADA coactivator invoking Retinoic Acid receptor response genes
- Binds hADA coactivator with perturbations in GAP pathways
- Induce mutations independent from p53 checkpoint effects
- Binds DNA 4 way junctions
Is Lichen Planus Precancerous?

• OLP is a relatively common disease, affecting 1 in 200 adults (.5%)(15,000,000)
• 1-2% of OLP patients develop SCCA (1:100, prevalence)
• Carcinoma may develop in the area of a lichenoid lesion or may arise in a nonlesional location
• Oral cancer is a rare disease affecting 35,000 Americans each year (35,000/298,000,000) or 1.2/10,000 (.012%). Incidence

Odds Ratios according to follow up period
83.3 for one year follow-up
8.3 for 10 year follow-up
4.2 for 20 year follow-up
Etiologic Factors in Oral Cancer

- Smoking Tobacco
- Alcohol
- Human Papillomavirus
- Low fruit/vegetable diet
- Lichen planus
HPV VACCINE
GARDASIL 9 is a vaccine indicated in girls and women 9 through 26 years of age for the prevention of the following diseases:

- Cervical, vulvar, vaginal, and anal cancer caused by Human Papillomavirus (HPV) types 16, 18, 31, 33, 45, 52, and 58. (1.1)
- Genital warts (condyloma acuminata) caused by HPV types 6 and 11. (1.1)

And the following precancerous or dysplastic lesions caused by HPV types 6, 11, 16, 18, 31, 33, 45, 52, and 58:

- Cervical intraepithelial neoplasia (CIN) grade 2/3 and cervical adenocarcinoma in situ (AIS). (1.1)
- Cervical intraepithelial neoplasia (CIN) grade 1. (1.1)
- Vulvar intraepithelial neoplasia (VIN) grade 2 and grade 3. (1.1)
- Vaginal intraepithelial neoplasia (VaIN) grade 2 and grade 3. (1.1)
- Anal intraepithelial neoplasia (AIN) grades 1, 2, and 3. (1.1)
GARDASIL

Age Regimen Schedule 9 through 14 years
2-dose 0, 6 to 12 months* 3-dose 0, 2, 6
months 15 through 26 years 3-dose 0, 2, 6
months *If the second dose is administered
earlier than 5 months after the first dose,
administer a third dose at least 4 months
after the second dose --------------------- 0.-
mL suspension for injection as a single-
dose vial and prefilled syringe.
HPV is an etiologic factor in a subset of Oral SCCAs

- Site: Tongue Base/Tonsillar Region
- Phenotype: Poorly differentiated
- pRb: Phosphorylated inactivated
- P53: Wild Type
- E6, E7: RNA and Protein expressed
- **Prognosis:** > than nonHPV/p53 CAs
- Metastases: HPV16 DNA present in histopathologically + and – lymph nodes

  - HPV 33, 59 have also been detected in tonsillar CAs
Well differentiated HPV16 negative

Poorly differentiated HPV 16 positive