Biology of Oral Cancer
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Oral Biology 720
July 27, 2008

Outline

• Review biology of oral cancer
  – Epidemiology/Clinic Aspect
  – Pathological Development
• Association of viral infection and oral cancer
  – Common oral cancer associated viruses
  – Human papillomavirus
    • Review its association with cancer
    • A case-control study
  • Review an article on HPV and oropharyngeal cancer

Epidemiology of Oral Cancer

• A subgroup of H&N malignancies
  – Lips, tongue, salivary glands, gingiva, floor of the mouth, oropharynx, buccal surfaces, and other intra-oral locations (ICD version 9)
  – 90% of OC is squamous cell carcinoma (OSCC)
    • 300,000 new cases WW(8th most common cancer)
    • 30,000 new cases in the US and 40,000 in the EU
    • Half of all malignancies in India and several Asia country
      – Tobacco and betel nut chewing

Risk Factors for OSCC

• Tobacco
• Alcohol
• Inherited (genetic) factors
  – 1.1 to 3.8 OR
  – Autosomal dominant?
  – Polymorphism of genes
    • Regulate nicotine and alcohol metabolism
      – CYP1A1
      – Glutathione S-transferase-M1
      – N-acetyltransferase-2
      – Alcohol dehydrogenase type 3 (ADH3)
    • Gene regulated cell cycle
      – Cyclin D

Tumor Staging & Prognosis

• TNM & pTNM
  – Tumor, Node, Metastasis
  – Does not provide accurate prognosis
  – Need more info
    • Loco-regional control
    • Extent of recurrence
    • Maximum tumor thickness
    • Differentiation grade & mode of invasion

Clinical & histopathological T classification

T1: Tumor 2 cm or less in greatest dimension
T2: Tumor more than 2 cm but not more than 4 cm in greatest dimension
T3: Tumor more than 4 cm in greatest dimension
T4: Tumor invades adjacent structures
Clinical & histopathological
N classification

NX: Regional lymph nodes cannot be assessed
N0: No regional lymph node metastasis
N1: Metastasis in a single ipsilateral lymph node, 3 cm or less in greatest dimension
N2: Metastasis in a single ipsilateral lymph node, more than 3 cm but not more than 6 cm in greatest dimension; or in multiple ipsilateral lymph nodes, none more than 6 cm in greatest dimension; or in bilateral or contralateral lymph nodes, none more than 6 cm in greatest dimension
N2a: Metastasis in a single ipsilateral lymph node, more than 3 cm but not more than 6 cm in greatest dimension
N2b: Metastasis in multiple ipsilateral lymph nodes, none more than 6 cm in greatest dimension
N2c: Metastasis in bilateral or contralateral lymph nodes, none more than 6 cm in greatest dimension
N3: Metastasis in a lymph node more than 6 cm in greatest dimension

Clinical & histopathological
M classification

MX: Distant metastasis cannot be assessed
M0: No distant metastasis
M1: Distant metastasis

Tumor Staging

<table>
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<tr>
<th>Stage</th>
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<tr>
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<td>Stage IVC</td>
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<td>M1</td>
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Therapy

- Stage I and II
  - Surgery or radiation
- Stage III and IV
  - Surgery followed by radiation
  - Multiple model protocols
    - Pre-op/post-op radiation and/or chemotherapy
    - OSCC is usually resistant to chemo.
      - Sensitivity test
        » Exfoliated cell or saliva

Pathological Development

- Multi-step model
  - Precancerous lesion
    - Benign hyperplasia/dysplasia
      - Leukoplakia: 4-18% of lesions become OSCC.
      - Lichen planus: 1-4% of lesions become OSCC.
      - Erythroplakia: 14-50% of lesion become OSCC.
  - Carcinoma in situ
  - Advanced lesion or Metastatic tumor

Theory of Field Cancerization

- Slaughter et al 1953
  - “Field of genetically altered cells”
    - Suppression of tumor suppressor genes
      - TP53, CDKN2A, pRb
    - These cells proliferate and expand to adjacent tissues causing further genomic damage.
    - A recurrent tumor occurs even with completed removal of the primary lesion.
Chromosomal Aberrations

- Genomic hybridization and microarray
- Number of aberrations increases with the progression of cancer.
- Precancerous/early lesion
  - 3p, 9p, 13p, and 17p
    - TP53 - 17p13
    - RB1- 13q14
    - CDKN2A – 9p21
    - Cyclin-dependent-kinase inhibitors – 9p21
    - FRA3B & FHIT- 3p14
- Advanced lesion
  - 5p11-22
  - 22q13
  - 1q
  - 10q
  - 21q

Oncogenes

- Increase malignancy potential
  - Promote unscheduled, aberrant proliferation
  - Override G-S, G-M and M checkpoints of cell cycle
  - Prevent apoptosis
  - Enable cellular survival under unfavorable conditions

Growth Factor Receptors

- ErbB family
  - ErbB1 or Her-1
    - Epidermal Growth Factor Receptor (EGFR)
    - Associated with Betel nut chewing
    - Gefitinib (an ErbB1 inhibitors
  - ErbB2 or Her-2 or Neu
    - Overexpress in oral lesion (> H&N)
    - Associated with worse prognosis

Small GTPases

- Ras family (H, K, and N)
  - K-ras mutations induce OC in mice.
  - Mutations occur 0-10% (US, EU, Japan).
  - India
    - H- and K-ras mutations is 28-35% of lesions.
    - Polymorphism of H-Ras increases OR to 1.6.
  - SH3 domain of Ras

Cyclin family

- Regulate cell cycle
- Cyclin D
  - Initiate G-S transition by phosphorylate Rb protein
  - CCND1
    - Worse prognosis
    - Single Nucleotide Polymorphism (SNP) is associated with susceptibility to OSCC.
- Cyclin A and B are also found.

Angiogenetic Factors

- Vascular growth factor (VEGF)
  - Expression of VEGF-C
    - Lymph node metastasis
  - Expression of FGF-4
    - VEGF receptor
    - Lymph node metastasis
Matrix Metalloproteinases (MMPs)

- Zn metalloenzymes
- Degradation of ECM
  - Gelatinases (MMP-1 and -13)
  - Stromelysin (MMP-3, -10, and -11)
  - Collagenases (MMP-1, and -13)
  - Membrane-bound MMPs (MT1-MMP)
- Malignant lesion: MMP-3, -9, -10, and -13
- Stroma: MMP-2 and -11
- MMP-2 and -9:
  - Invasive potential
  - Alcohol consumption
- MMP-2: metastasis

Tissue inhibitors of MMPs (TIMPs)

- Inhibit MMPs by binding to MMPs.
- TIMP-1 and -2
  - Are homologous to erythroid potentiating (EPA) factors
  - Promote growth of erythroid precursor cells
- TIMP-2
  - Local recurrence and poor prognosis

Tumor Suppressor Genes

- Prevent cells from acquiring malignant characteristics by
  - Regulation of discrete check-points during cell cycle
  - Monitoring of DNA replication and mitosis
- Activated by cell stress and other insults

Retinoblastoma Protein (Rb)

- Hypo-phosphorylated forms of Rb proteins
  - Bind and inactivate E2F transcription factor
  - Inhibit G to S transition
- Rb expression
  - Suppressed
  - 70% of oral tumors
  - 64% of premalignant lesions.
  - ½ of OC has no pRb expression.
  - 20% express only inactive hyper-phosphorylated form.
- Disruption of at least one component in pRb network
  - 84% of premalignant lesions
  - 90% of OSCC

Cyclin dependent kinase inhibitors (CDKIs)

- Known cancer target
- Inhibit pRb phosphorylation
  - CDKN2A locates in 9p21 encodes p16INK4A.
  - Suppression of p16 expression
    - 83% of oral tumors
    - ~60% of pre-malignant lesions
  - Alternative spliced variant- p14ARF is also suppression.

p53 tumor suppression system

- p53 protein stops proliferation by arresting cell cycle and inducing apoptosis.
- Heterogeneity of TP53 (17p13) is common in OSCC.
- 57% of OSCC has an up-regulation of p53
Viral Infection and OSCC

- Human Papillomavirus (HPV)
- Epstein-Barr Virus (EBV)
- Hepatitis C Virus (HCV)

Epstein-Bar Virus (EBV)

- Belongs to Herpesvirus family.
- Is associated with malignancy of B cell.
- Its association with OSSC is inconclusive.
  - More frequently found in OSCC & LP
  - LMP1 protein found in OSCC
  - Inconsistent reports

Hepatitis C Virus (HCV)

- Oral verrucous and OSCC found in HCV-infected patients.
- HCV infection found in oral LP.
  - May be associated with oral LP development.
  - Only 1-2% of LP becomes OSCC.

Human Papillomaviruses (HPVs)

- Host-specific DNA viruses
- Papillomaviridae family
- Specifically infect epithelial cell with an intra-nuclear mode of replication
- Capsid proteins
  - L1: 54 KDa
  - L2: 76 KDa
  - Target tx
- DNA
  - GC content – human
    - 42.6-50% (42-43% human)
  - E, L, and LCR
    - ORF (E + L)
Genotypes of HPV

- Use level of homology of nucleotide sequence in the genomic regions. (e.g. E6, E7, and L1)
- > 100 genotypes isolated
  - http://hpv-web.lanl.gov/
    - < 90% new type
    - 90-98% sub-type
    - ≥ 98% variant

Types of HPV based on their Oncogenesis Potential

- High Risk (HR)
  - Malignant transformation
    - Cervical and oral cancers
    - 16, 18, 31, 33, 35, 58
- Low Risk (LR)
  - Benign lesions
    - Papilloma lesions
    - 2, 4, 6, 11, 13, 32

HPV infection

- Southern Blotting (SB)
  - Isolate DNA, digest, blot, and use labeled specific probes
- In situ Hybridization (ISH)
  - Probe directly to the tissue specimen without DNA isolation
- Polymerase Chain Reaction (PCR)
  - Amplify DNA with specific primers, and therefore more sensitive SB and ISH.

HPV and Oncogenesis

- pE6 + p53
  - p53 degradation
- pE7 + p105Rb
  - Alter regulation of cell cycle through E2F

HPV & Oral Premalignant lesions

Mostly HPV16 or 18
HPV & Oral Premalignant Lesions

Problems in HPV-Oral Cancer Studies

- HPV detection technique
- HPV virulence
  - HR or LR
  - Expression of viral oncoproteins
    - E6, E7
- Other factors
  - Smoking
  - Alcohol abuse
- Tumor staging, recurrent and metastatic potential
  - Not always correlated with HPV (HR) infection and oncoprotein expression
  - Most HPV related tumors:
    - Based of the tongue and tonsillar areas.

Questions

Thank You

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