Strategies for Prevention of HCC
Molecular Epidemiology

• Identify risk factors and outcome

• Biomarkers
  • Carcinogen-macromolecular adducts
  • Normal DNA sequence variants
  • Mutations in target genes

• Measure in urine, serum or tissue
  • Immunoassays
  • GC/MS, LC/MS
  • Florescence spectometry
HCC Epidemiology

- Annual new cases ~600,000
- ~600,000 annual deaths
  - 80% burden in Asia and sub-Saharan Africa
  - 300,000+ cases in People’s Republic of China
- High risk areas early age of onset 20’s
- Low risk areas early age of onset 50’s
HCC Epidemiology

• Main causes in high risk areas
  – HBV infection
  – Aflatoxins in diet

• Synergism leading to increased risk

Impact on HCC incidence

HBV vaccination

aflatoxin exposure
HCC Epidemiology: Aflatoxin Studies

• Taiwan\(^1\): BsAg+ males with HCC compared to control subjects
  • OR = 2.8 detectable vs. nondetectable aflatoxin metabolites
  • OR = 5.5 high vs. low urinary metabolite levels

• Shanghai\(^2\): relative risk for HCC with presence aflatoxin metabolites = 3.8

Aflatoxins

- Produced by fungi
  - 1960 outbreak of “Turkey ‘X’ disease” in UK
    - Aspergillus flavus
- Common in corn, peanuts, fermented soy products

![Aflatoxin B₁](image)

Cytochrome P450

Active DNA-modifying agent

Figure by MIT OCW.
HCC Prevention/Intervention

• Primary
  – Vaccination
  – Reduced contamination

• Secondary
  – Pharmaceuticals
  – Natural products
HCC Prevention/Intervention

- **Hepatitis B Virus**
  - HBsAg (serum)
  - Insertional Mutagenesis
  - X-Gene Mutations
  - Chronic Inflammation
  - Antiviral drugs
  - Green tea polyphenols, COX-2 inhibitors, ITCs, vitamin K analogs, retinoids
  - Cell Proliferation

- **Aflatoxin B₁**
  - Chlorophyllin
  - Reactive Intermediates
    - Oltipraz, ITCs
  - Promutagenic DNA Lesions
    - Aflatoxin-Mercapturic Acid (urine)
  - p53 Gene Mutations (G:T-T:A transversions)
    - Aflatoxin-N⁷-guanine (urine)
    - 249⁶⁶ Mutations (serum)
  - Selective Clonal Expansion and p53 Allelic Deletion
  - Chronic Hepatitis and/or Cirrhosis
    - Oltipraz
    - Hepatocellular Carcinoma

Figure by MIT OCW.

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Primary Interventions

• HBV vaccination
  – Taiwan HCC cases in 6-14 year olds
    • Born 1981-1986 = 0.70
    • Born 1986-1990 = 0.57
    • Born 1990-1994 = 0.36

• Reduction of aflatoxins in food

Secondary Intervention

Oltipraz

Isothiocyanates

R – N = C = S

Chlorophyllin

Polyphenols

R

May 2nd, 2005

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Figures by MIT OCW.
Secondary Intervention: Oltipraz

- Oltipraz
  - Induces phase 2 enzymes
  - Inhibits phase 1 enzymes
- Higher doses (500mg+) not more effective at induction or inhibition than lower doses (125mg and 250mg)
Mechanism of Oltipraz

Secondary Intervention: Oltipraz

• Phase IIa intervention trial
  – Feasibility of biomarker measurements
  – Dose response
  – Tolerance/effectiveness longer term exposure
  – Chronic toxicity

Secondary Intervention: Oltipraz

Location: Dazin Township, Qidong, People’s Republic of China

- Randomized, placebo-controlled, double blind
- 240 adults without history of chronic disease
- Detectable serum aflatoxin-albumin adducts
- 3 intervention groups
  1) Placebo
  2) 125mg once daily
  3) 500mg once weekly

Secondary Intervention: Oltipraz

- **500mg weekly after 1 month**
  - 51% decrease median levels aflatoxin $M_1$ excretion
  - No effect on aflatoxin-mercapturic acid
  - Inhibits activation

- **250mg daily after 1 month**
  - 2.6-fold increase in median levels of aflatoxin-mercapturic acid
  - Modest effect on aflatoxin $M_1$ levels
  - Increase phase 2 conjugation

Secondary Intervention: Oltipraz

• Ongoing follow-up phase IIb trial
  – Sustained expression enhancement of aflatoxin detoxification enzymes
  – 250mg versus 500mg once weekly for 1 year
  – Measuring multiple biomarkers for mechanisms of action
Secondary Intervention: Chlorophyllin

- Mixture of sodium-copper salts of chlorophyll
- OTC drug
  - Wound healing accelerant
  - Controls body, fecal and urinary odor
- *In vitro* and *in vivo* antimutagen in short-term genotoxicity assays
Secondary Intervention: Chlorophyllin

- Complexes with aflatoxin B1
- Reduction in bioavailability
- Needs molar excesses to carcinogen for efficacy
- *In vitro* inhibitor of cytochrome P450 enzymes
- Antioxidant-reduction in lipid peroxidation
Secondary Intervention: Chlorophyllin

• Chemoprevention study in Qidong
  – 180 healthy adults
  – 100mg chlorophyllin or placebo 3-times daily for 4 months
  – Endpoint of modulated aflatoxin-N7-guanine adducts in urine after 3 months

• Resulted in 55% decrease in median urinary adduct levels

Secondary Intervention: Isothiocynates

Source: Family Cruciferae (mustards), Genus Brassica (cauliflower, Brussels sprouts, broccoli, cabbage)

- Lower cancer rates in individuals consuming high levels of yellow and green vegetables
  - Isothiocyanates
  - Particularly glucosinolate precursors
  - Sulforaphane induces phase 2 enzymes in rats (glucoraphanin is precursor)
Secondary Intervention: Polyphenols

Source: Green tea

- Inverse association of consumption versus risk and development of cancer
- Green tea-derived polyphenols (ongoing study)
  - Reduce aflatoxin M$_2$ excretion
  - Increase aflatoxin-mercapturic acid excretion
  - Reduced 8-oxo-deoxyguanosine
Outlook: Primary Interventions

• HBV vaccination
  – Only benefits younger generations
  – Vertical transmission not prevented
• Reduced food contamination
  – Requires infrastructure for production, processing and distribution
  – Monitoring mycotoxins $$
  – Not feasible in developing countries
Outlook: Pharmaceutical Chemoprevention

• Not practical for populations at highest risk
  – SE Asia, China, Africa
• First-generation (oltipraz) expensive
• 2nd and 3rd generation dithiolethiones
  – Cheaper
  – 10-fold increase in potency over oltipraz
  – Ongoing safety evaluations
• Long-term costs potentially high, chronic treatment
Outlook: Natural Products

• Practical for populations at highest risk
  – SE Asia, China, Africa
• Inexpensive, diet-based
• Long-term compliance better
• Immediate impact
Potential Impact

- Reduction of aflatoxin-N7-guanine
  - Reduced risk HCC in animals
  - Increased latency period
- Decreased aflatoxin exposure in Beijing correlated with later onset of HCC

Questions?