Pushing Forward the Biological Understanding and Diagnostic Applications of Circulating DNA

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Prenatal Diagnosis

Amniocentesis
Needle in a Haystack
Y chr

Case number 17 22 5 39 32 36 38

Maternal serum

Lo et al. Lancet 1997; 350: 485
Fetal DNA: 15%
Rapid Clearance After Delivery

Case S4

Case S5

SRY (copies/ml)

Time (min)
Sex-linked disorders
Blood Group Testing

A⁺ B⁺ AB⁺ 0⁺
A⁻ B⁻ AB⁻ 0⁻
Down Syndrome
A challenging problem

Fetal DNA

Maternal DNA

Plasma
Single Molecule (Digital) Testing
Accuracy: 99.7%

Chiu et al BMJ 2011
Millions of cases performed in 90 countries
GOAL
BEYOND DOWN SYNDROME
BEYOND DOWN SYNDROME

FETAL GENOME
Genome fragmented

Millions of pieces

Mixed with maternal DNA

TECHNICALLY CHALLENGING
Father

Mother
Mother
Couple: Carriers for beta-thalassemia
12 weeks pregnant
• ~4 billion DNA molecules sequenced
• ~65-fold genome coverage
Fetus is a carrier of beta-thalassemia
Liquid Biopsy for Cancer
Nasopharyngeal Cancer (NPC)
Geographical Prevalence
Plasma EBV DNA concentration (copies/mL)

NPC patients

Healthy controls

Lo et al Cancer Res 1999
Characteristics of Plasma EBV DNA

DNA Fragments vs Virions
Plasma EBV DNA: Size Profiling

Fractional plasma EBV DNA concentration (relative to the 82 bp amplicon)

Size of amplicon (bp)

- 100% 13.0%
- 9.6% 2.8%
- 0.9% 0.6%
- 0.3% 0.2%

Chan et al. Cancer Res 2003
Short Half-Life
Radiotherapy

Half-life = 4 d

Surgery

Half-life = 1.5 h

To et al. Clin Cancer Res 2003
Clinical Monitoring
Progression

![Graph showing serum EBV DNA levels over time with annotations for progression markers such as bone metastases (bone mets), cord compression, and after palliative RT. The graph includes data points for CR and RT, with axis labels for period of follow-up (days) on the x-axis and serum EBV DNA (copies/ml) on the y-axis. The graph is labeled Lo et al. Cancer Res 1999.]
Continuous Remission

Lo et al. Cancer Res 1999
Early Detection?
76% of NPC cases in Hong Kong present in Stages III and IV
NPC Screening by Plasma EBV DNA

• 20,000 subjects over 3 years
• Males: 40-60 years
• Detecting NPC at earlier stages
Enrolment to the study

Positive plasma EBV DNA

4 weeks

Persistently positive plasma EBV DNA on two occasions

Plasma EBV DNA test

FU plasma EBV DNA test

Persistently positive plasma EBV DNA on two occasions

Nasal endoscopy and MRI assessment

Yearly follow-up
- development of NPC
- development of other cancers

Timeline
Subject recruitment

Weekly community visits
Results

• 20,174 subjects recruited and screened
• 1,112 (5.5%) subjects with first plasma EBV DNA positive
• 309 (1.5%) subjects with two consecutive positive plasma EBV DNA tests
• 34 cases of NPC identified
• Positive Predictive Value: 11%
Stage Distribution

NPC cases identified by screening

Stage Distribution

Stage
- I
- II
- III
- IV

NPC without screening
Stage Distribution

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<th>Stage Distribution</th>
<th>NPC without screening</th>
<th>NPC cases identified by screening</th>
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<td>NPC cases identified</td>
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Percentage distribution:
- Stage I: 100%
- Stage II: 80%
- Stage III: 80%
- Stage IV: 20%

NPC without screening:
- Stage I: 100%
- Stage II: 40%
- Stage III: 30%
- Stage IV: 20%

NPC cases identified by screening:
- Stage I: 80%
- Stage II: 40%
- Stage III: 30%
- Stage IV: 20%
Progression-free Survival

Patients identified by screening

Patients in historical cohort

Probability of Survival (%)

Hazard ratio, 0.10 (95% CI, 0.05–0.18)

Months since Start of Treatment
Analysis of Plasma Epstein–Barr Virus DNA to Screen for Nasopharyngeal Cancer

NPC cell

~50 copies of EBV genomes

each containing

~10 copies of PCR target
Nasopharyngeal cancer
Other Cancers
Summary

• Plasma nucleic acids represent a treasure trove for molecular diagnostics
• Era of non-invasive prenatal testing is here
• Concepts applicable to cancer liquid biopsies
• Early detection of cancer
Thank You!