Using Mutations to Find the Needle in the Haystack

Jason H. Bielas
Assistant Member, Molecular Diagnostics, Fred Hutchinson Cancer Research Center
Cancers Clonally Accumulate Somatic Mutations

Random mutations fuel tumor progression

Predict speed of progression/resistance to therapy
Mitochondria

- Energy powerhouses
- Have their own DNA
- Mitochondrial DNA is replicated by a separate enzyme to nuclear DNA
Clonal mtDNA Mutations in Cancer

- Colorectal Carcinoma
- Adenoma
- Prostate Cancer
Mitochondria Homoplasmic Mutations

(a) Mutagenesis

Wild-type

CATATCGATATA
GTATAGCTATAT

Mutant

CATATCXATATA
GTATAGXTATAT

(b) Intramitochondrial

(c) Intracellular

(d) Intercellular
## Detecting Mutations

### Method

- SSCP
- ASA, PASA
- Mismatched PCR
- PNA clamping, DGGE
- NextGen Sequencing
- Block-PCR, CDCE, $M_2B_2$
- MS-PCR, RSM
- ACD-PCR, Gap-LCR, MutEx/PCR/SNuPE
- MAMA, 2X CDCE
- PCR/RFLP,
- RFLP/PCR

### Sensitivity

<table>
<thead>
<tr>
<th>Method</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>NextGen Sequencing</td>
<td>$10^1$</td>
</tr>
<tr>
<td>A → T</td>
<td>$10^2$</td>
</tr>
<tr>
<td></td>
<td>$10^3$</td>
</tr>
<tr>
<td></td>
<td>$10^4$</td>
</tr>
<tr>
<td></td>
<td>$10^5$</td>
</tr>
<tr>
<td></td>
<td>$10^6$</td>
</tr>
<tr>
<td></td>
<td>$10^7$</td>
</tr>
<tr>
<td></td>
<td>$10^8$</td>
</tr>
<tr>
<td></td>
<td>$10^9$</td>
</tr>
</tbody>
</table>
mtDNA Mutation as Cancer Biomarkers

Mitochondrial separation and mtDNA isolation

DNA

Plasma

Dynabead magnetic mtDNA enrichment

Genotypic-selection (restriction digestion)

Frequency of mutant ctmtDNA

Frequency of CTCs

GAG ATC G T

G ACC

LCM-isolated cancer cells

Identify somatic mutations
Predict the Future

at 13-56d follow-up
• mtDNA mutations may help stratify cancer patients that progress.

• We can use mitochondrial mutations to mark tumor cells

• Circulating tumor cells (and DNA) can be used to predict and monitor for recurrence

• Early detection of malignant cancers via mutation detection is possible
Acknowledgements

Nuclear DNA
Mary Bronner (Cleveland Clinic)
Eddie Fox (U College Dublin)
Keith Loeb (FHCRC)
Jacintha O’ Sullivan (U College Dublin)
Brian Rubin (Cleveland Clinic)
John Silber (UW)
Elizabeth Swisher (UW)

Mitochondrial DNA
Tina Albertson (UW)
Irv Bernstein (FHCRC)
Vilhelm A. Bohr (NIA)
Warren Ladiges (UW)
John Morton (UW)
Tomas Prolla (UW-Madison)
Peter Rabinovitch (UW)
Nadja Souza-Pinto (NIA)
Roland Walter (FHCRC)

Prostate
Beatrice Knudsen (FHCRC)
Colm Morrissey (UW)
Elahe Mostaghel (FHCRC)
Pete Nelson (FHCRC)
Lawrence True (UW)
Bob Vessella (UW)

Support
Pacific NW Prostate Cancer SPORE (P50-CA97186)
NIH/NCI 5 P30 CA015704-34, NIH ONES R01
Ellison Medical Foundation
DoD