The use of diagnostic FFPE material in cancer epidemiology research

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• Who we are
• What we do
• Why FFPE (Formalin Fixed Paraffin Embedded)
• Challenges of using FFPE
• Future uses of FFPE
• Gain Support for collections in the future
Pathology Epidemiology, DNA, Informatics and Genetics: a Research Enabling Enterprise

(http://www.pedigree.org.au)
Pedigree Cancer Study

Endeavours to generate evidence so that cancer genomics can be used to prevent cancer through:

- Leading and contributing to, the discovery of new genomic and molecular advances that help identify people at genetic risk of cancer
- Using epidemiological and epigenetic data to find the environmental and lifestyle exposures relevant to people at genetic risk of cancer
- Develop novel statistic and high performance computing methods
- Nurturing and building the expert multi-disciplinary research team
- Curating and building all aspects of the resource
- Growing national and international collaborators
The Bio-repository

Bio-Specimen Processing
- Blood
- Saliva
- Histology

Sample Storage
- 1 Million+ Frozen Blood Fractions
- 35 000 Saliva
- 10 000+ FFPE Cases (slides)
- 150 000 Guthrie Cards (duplicates in 2 locations)

Tissue Culture
- Cell Lines
- Active

Resource for Research
ABC Australian Breakthrough Cancer Study

Can I take part?
You can take part if you're an Australian resident, 40 to 74 years of age, and have never been diagnosed with cancer.

Australian Resident  
Between 40 and 74 years of age  
Have never been diagnosed with cancer  
Yes

You don’t have to have cancer to fight cancer.  
Register now

• 11 000 / 20 000 blood samples
• 35 000 / 50 000+ saliva samples
• Future – New Incidents of Cancer

www.abcstudy.com.au
Study Cycle

Study Development

Funding - Australia and International (NIH)

Ethics - Hrec
- Site Specific Ethics

Participants - Joining a Study
- Cancer Registries
- Family Cancer Clinics

Recruitment (up to 1 year)
- Informed Consent
- Questionnaire
  - Lifestyle
  - Family History
  - Clinical History

Blood / Saliva Collection (Base Line)

± Tissue Collection

Pedigree

± Pathology Review

Molecular Testing

Data / Publication

Translational Pathology Outcomes

Extension

Follow Up (FUP) 5 years
Study Cycle – Follow Up

**Study Modification**

**Funding** - Australia and International (NIH)

**Ethics**
- Hrec
- Site Specific Ethics

**Participants** - Targeted Family Members

**Re- Recruitment (up to 1 year)**
- New Informed Consent
- Questionnaire
  - Lifestyle
  - Family History
  - Clinical History

**Blood /Saliva Collection (FUP)**

**New Incident Tissue Collection**
- Cancer Registry Searches

**Death Registry Searches**

**Pedigree Updates**

**Pathology Review**

**Molecular Testing**

**Data / Publication**

**Translational Pathology Outcomes**

**Extension**

**Follow Up (FUP2)**
Blood
Saliva
“Normal” Tissue

GERM-LINE MUTATIONS

Entire organism carries the mutation

Half of gametes carry mutation

SOMATIC MUTATIONS

Parental Gametes
Embryo
Organism
Gametes of Offspring

Somatic mutation

Patch of affected area

None of gametes carry mutation
• Epigenetic Factors
  – Methylation

• Genetic Factors
  – New Genetic Variants

• IHC
  – Screening Tool
  – Prognostic Markers
Translational Pathology - Outcomes

- FFPE Tumour Classification
  - Prognosis
  - Risk Measurements
  - Treatment Options

- Somatic Tumour Testing

- Germline Testing
  - Carriers
    - Intensive Screening
  - Non Carriers
    - Less Intensive Screening

- Blood/Saliva Predictive Testing
  - Risk Measurements
  - Prevalence
  - Penetrance

Other Cancers

Risk Measurements

Prevalence

Penetrance
# Molecular Subtypes of Breast Cancer

<table>
<thead>
<tr>
<th>Molecular Subtype</th>
<th>Luminal (A and B)</th>
<th>HER2</th>
<th>Basal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genetic profile</td>
<td>Luminal CKs and ER-related genes (A&gt;B)</td>
<td>HER2-related genes</td>
<td>Basal CKs</td>
</tr>
<tr>
<td>Histologic correlates</td>
<td>A: Lower-grade ER+</td>
<td>B: Higher-grade ER+</td>
<td>High-grade, apocrine features</td>
</tr>
<tr>
<td>Surrogate markers</td>
<td>A: Strong ER+, PR+, HER2+, low Ki67</td>
<td>B: Weaker ER+, PR+, HER2+, Ki67</td>
<td>HER2+, ER+/--</td>
</tr>
<tr>
<td>CK5/6+:--</td>
<td>ESRF+--</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prognosis</td>
<td>Good</td>
<td>Intermediate</td>
<td>Worse</td>
</tr>
<tr>
<td>Response to chemotherapy</td>
<td>Lower</td>
<td>Intermediate</td>
<td>Higher</td>
</tr>
<tr>
<td>Targeted therapies</td>
<td>Hormone therapies</td>
<td>HER2-targeted therapies</td>
<td>Currently investigational</td>
</tr>
</tbody>
</table>

**Luminal A 40%**
**Luminal B 20%**
**Her2 Enriched 15-20%**
**Basal 15-20%**

**Basal Like**

**Triple negative (BRCA1 80%)**

- **70%**
- Basal-like tumors
- Triple negative tumors
Expression-based classification of CRCs from 4,151 patients

**CMS1 – MSI immune**
- Hypermutated, MSI-High and strong immune

**CMS2 – canonical**
- Epithelial, marked WNT and MYC signalling

**CMS3 – Metabolic**
- Epithelial, metabolic dysregulation

**CMS4 – Mesenchymal**
- TGF-β activation, stromal invasion, angiogenesis
1) MMR Deficient
2) Immune Rich

Germline Testing
MSI High

Carrier

Treatment - Check Point Inhibitor Therapy
• Pembrolizumab (PDL1)

Present                   Absent
PDL-1 Normal Tissue      Cancer
Collection Challenges

- Availability
- Quality
- Storage
- Cost
Collection Challenges

Availability

- Retention times
  - Long cohorts
  - Pedigrees
- Remote archiving
  - Storing and not accessing
- Workload
- Amalgamated / closed laboratories / Custodians?
- Amount of tissue remaining in blocks
- Resistance to access
  - High price to deter
  - Consented / ethics
Collection Issues

Quality

• DNA
• Antigenicity

– Storage time
– Storage Conditions
  - Transport
  - Temperature
  - Sunlight Exposure UV
– Oxidation
  - air
– Hydrolysis
  - Endogenous water
  - Exogenous water e.g. 4°C Fridge
– Tissue processing time
  - Inadequate
  - Prolonged
  - Fixation time / type

1 Fresh cut sections from Block

2 Sections stored 4 to -20°C
Collection Challenges

Storage
  – Bundling
  – Dark
  – Air tight
  – 4°C

• Lab Storage
  – Limited space
  – Cost
Cost

- Cost of collection is rising
- Impact on existing funding
  - Budgets
- Funding decreasing / harder
- Cost recovery
The Future of FFPE Testing

- ?? Independent Repository
- New diagnostic tests
  - Molecular technologies – Cheaper Better
  - Molecular Testing / IHC
  - Research tests = Diagnostic tests = Revenue for laboratories
- IHC to better define areas of interest
  - Tumour heterogeneity
- Collecting more “Normal” and other pre-cancers lesions
  - Predictive tests for patients and families
  - Clinicians driving change
“Normal” Colon from MMR Deficient Carriers

MSH2

MSH6
Future - Using IHC for Macro/Micro-Dissection

Laser Capture Micro-Dissection

Ducts or Crypts
The Victorian Cancer Registry

- 1982-2015
- Cancer prevalence statistics

http://vcrdata.cancervic.org.au/vs
Concluding

- Focus on FFPE
  - Tumour
    - New gene variants
  - Normal
- Feedback on our requesting
- What can we give back to keep your labs invested
- Our participants want their tissues included in our research.
- Thank you for your contributions to our studies
- Researchers need your support
Acknowledgements

• Our Participants
• Funding bodies
• All laboratories that have provided tissue for our studies