Dissecting Intra-Tumour Heterogeneity: Patterns, Dynamics and Clinical Implications

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Personalized Cancer Medicine

Sequencing (Exome/Genome/CNVs)

Prognosis

Prediction of treatment response

Tumor
Intra-Tumour Heterogeneity in Clear Cell Renal Cell Carcinomas (ccRCCs)
Morphological Heterogeneity in clear cell Renal Cell Carcinoma (ccRCC)

Nephrectomy Specimen
Construction of regional mutation heatmaps

Validation of all non-synonymous mutations at 400x

Exome sequencing with 70-100x depth per region

Multi-region harvesting of nephrectomy specimens

Non-synonymous somatic mutation calling

ccRCC Multi-Region Exome Sequencing (M-Seq)

- 10 ccRCC cases sequenced and analysed to date
- 8 regions per tumour on average
- all VHL (-/-)
Spatially Separated Somatic Mutations Revealed by Multi-Region Exome Sequencing

Is a Single Tumour Biopsy Representative of the Entire Tumor Bulk?

Total somatic mutational load: 128 (100%)
Average somatic mutations per biopsy: 70 (55%)
Is this Genetic Intra-Tumour Heterogeneity Functionally Relevant
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Is this Genetic Intra-Tumour Heterogeneity Functionally Relevant?

5 independent mutations in 2 histone modifying enzymes with distinct spatial distributions
mRNA expression profiling and unsupervised clustering of transcripts in Brannon ccA/ccB dataset

Brannon, Genes Cancer, 2010
Intra-Regional Heterogeneity

Based on variant allele frequencies from deep sequencing validation

Gerlinger et al, Nat Genetics 2014
Mutation Detection by Number of Biopsies
Relevance of ITH for Driver Mutation Prevalence Estimates in ccRCC

<table>
<thead>
<tr>
<th>Gene</th>
<th>Prevalence in TCGA samples (n=102 samples)</th>
<th>Prevalence in all M-seq samples (n=79 samples)</th>
<th>Prevalence in patients based on M-seq (n=10 patients)</th>
<th>Prevalence patients / Prevalence biopsies</th>
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<tbody>
<tr>
<td>PBRM1</td>
<td>42%</td>
<td>39%</td>
<td>60%</td>
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<tr>
<td>SETD2</td>
<td>18%</td>
<td>27%</td>
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<td>11%</td>
<td>10%</td>
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<tr>
<td>TSC2</td>
<td>2%</td>
<td>4%</td>
<td>10%</td>
<td>2.6</td>
</tr>
</tbody>
</table>

Gerlinger et al, Nat Genetics 2014
Copy Number Driver Heterogeneity

Gerlinger et al, Nat Genetics 2014
Conclusions: ITH and Tumour Biological Concepts

• ITH and branched evolution are common in advanced ccRCCs
• Single biopsies cannot detect this complex subclonal architecture
• Obligatory truncal events: VHL mutation/methylation and chromosome 3p loss
• Other drivers tend to be located on branches
• Mutations in these genes do not appear to define natural subtypes of VHL-/- ccRCCs
• If all ccRCCs are born equal, yet patient outcomes are diverse: subclonal driver are likely to influence outcome
Evolution Generates Diversity
Spatially Separated Genetic Heterogeneity in Other Tumour Types
High Grade Serous Ovarian Cancer

Bashashati, J Path 2013
Pancreatic Cancer: Spatially Separated Tumour Subclones in Metastatic Sites

Campbell, Nature 2010
Intermixed Genetic Heterogeneity
ITH in Triple Negative Breast Cancer

Shah, Nature 2012
Clinical relevance of subclonal mutations – a key question in next-gen cancer sequencing

Shah, Nature 2012
ITH in Glioblastoma Multiforme

Snuderl et al, Cancer Cell 2011
ITH and Representation of AML Subclones in Bone Marrow and Peripheral Bloods

Figure 1. Mutational and Subclonal Comparison of Peripheral Blood and Bone Marrow Leukemia Samples

Klco et al. Cancer Cell 2014
AML Subclones can be Functionally Distinct

Klco et al. Cancer Cell 2014
Genetic Heterogeneity at the Single Cell Level

Xu, Cell 2012
Navin, Nature 2011
Clinically Dominant Tumour Subclones
Clonal Origin of a Lethal Metastatic Prostate Cancer

Haffner et al. JCI 2013
Clonal Origin of a Follicular Lymphoma Relapses and of Transformation

Okoson et al, Nature Genetics 2013
The Evolution of Cancer Drug Resistance
KRAS Mutation Status of Primary-Metastasis Pairs in Colorectal Cancer

Vakiani, JCO 2012
Acquired resistance mutations to EGFR targeted therapy in CRC

Fig. 6. Heat map of acquired resistance mutations to EGFR blockade in ctDNA from patients with metastatic CRC.

Bettegowda et al, STM 2014
Temporal Heterogeneity: Heterogeneity in Metastatic Cancers Through Circulating Tumour DNA

Diaz, Nature, June 2012
Temporal ITH and Clonal Responses to Drug Therapy in Multiple Myeloma
Intra-Tumour Heterogeneity and Evolutionary Dynamics as Novel Biomarkers
Clonal/Genetic Diversity Predicts Barrett’s Oesophagus Progression to Adenocarcinoma

Maley et al, Nature Genetics 2006
Clinical Impact of Subclonal Mutations in CLL

Landau, Cell 2013
Clinical Impact of Subclonal Mutations in CLL

B

\[ P = 0.015 \]

% alive without retreatment

No evolution (n = 2)

+ evolution (n = 10)

Months from first therapy

C

\[ P = 0.041 \]

Subclonal driver absent (n = 4)

Subclonal driver present (n = 8)

Landau, Cell 2013
Conclusions: Cancer Evolution Models

Linear evolution model

Branched evolution model
Conclusions: Treatment Strategies for Heterogeneous Cancers

Yap, Gerlinger et al, STM 2012
Conclusions: Implications of Genetic Tumour Heterogeneity

- Sampling bias due to spatial separation of subclones
- Intermixed subclones can be detected by deep seq
- Clinical relevance of subclones (prognostic and predictive)
- Can we refine prediction by monitoring subclonal dynamics
- Define common evolutionary paths of cancer entities
- Relevance of ITH for therapeutic targeting: targeting of trunk aberrations
- Additional complexity: non-genetic heterogeneity
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