Proteomic investigation of intra-tumor heterogeneity

Tiannan Guo, MD, PhD

Ruedi Aebersold lab

Institute of Molecular Systems Biology

ETH Zurich

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Personalized/precision medicine

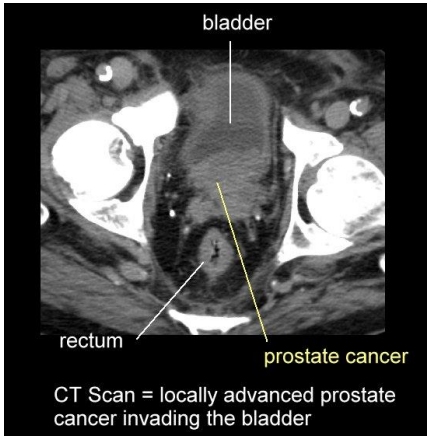
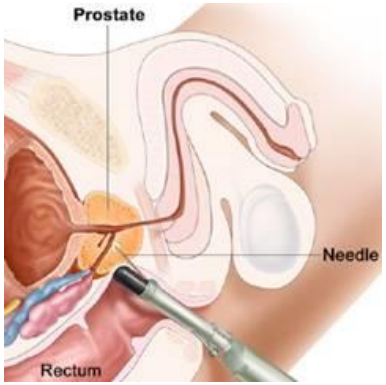


Image credit:
http://www.aboutcancer.com/prostate_anatomy_images_ct.htm

Tissue needle biopsy



Characterization of bio-molecules

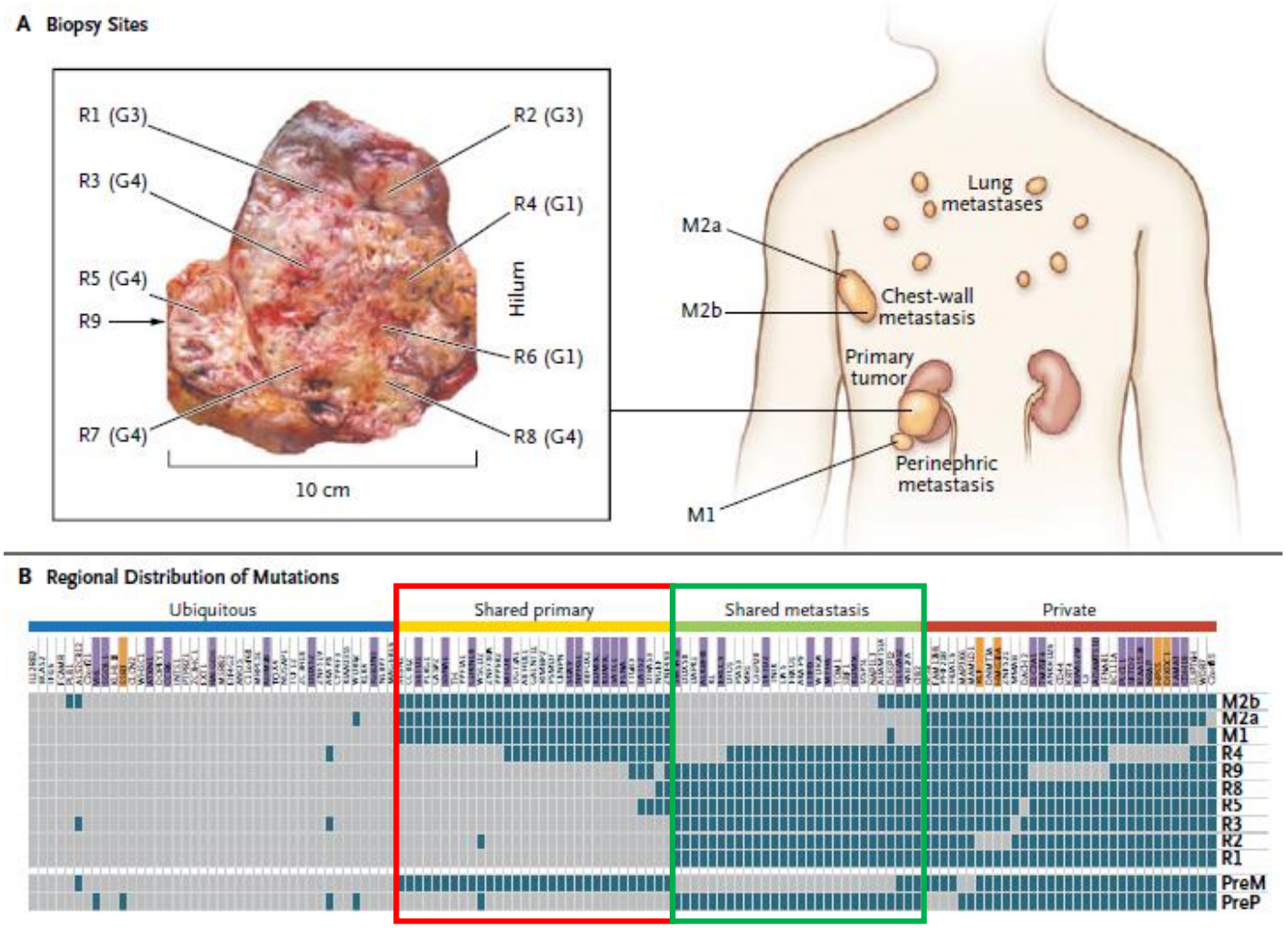
- NGS
- proteomics;
- metabolomics

Query knowledgebase

Treatment selection

Assumption: tumor is homogeneous

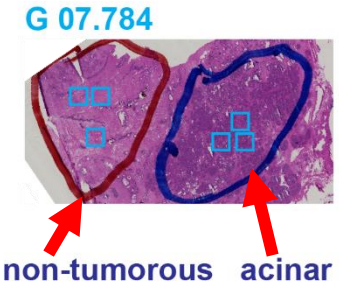
Tumor is genetically not homogeneous

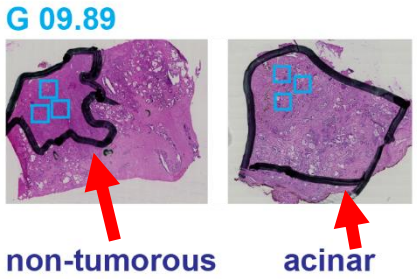


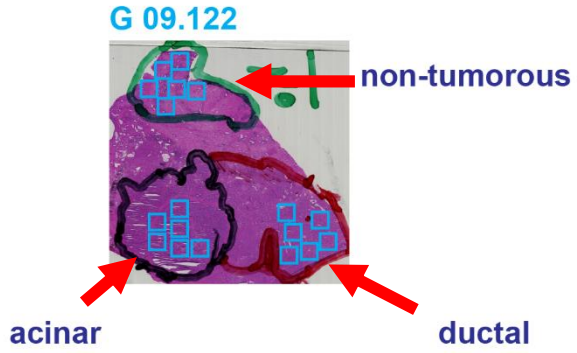
What about proteins?

Marco Gerlinger, et al. New England Journal of Medicine. 2012.

Experimental design







punchID	patientID	Tissue area	Replicates ID
1	1	normal	1
2			2
3			3
4		acinar	1
5			2
6			3
7	2	normal	1
8			2
9			3
10		acinar	1
11			2
12			3
13	3	normal	1
14			2
15			3
16			4
17			5
18			6
19		ductal	1
20			2
21			3
22			4
23			5
24			6
25		acinar	1
26			2
27			3
28			4
29			5
30			6

x2 =60 samples

Technical hurdles

- Small sample amount
- Large number of samples to be analyzed
- Reproducible quantification
- Tissues are “dirty” samples

Our solution

PCT-SWATH: Fast conversion from biological samples to digital data

Biological samples → BIG Data → Knowledge

Pressure cycling technology (PCT)-assisted tissue lysis and digestion

tissue biopsy
~1mg

→

All procedures
in single tube

→

Batch processing
Minimize variations

→

Pressure cycling technology
fast and efficient digestion

→

Yield:

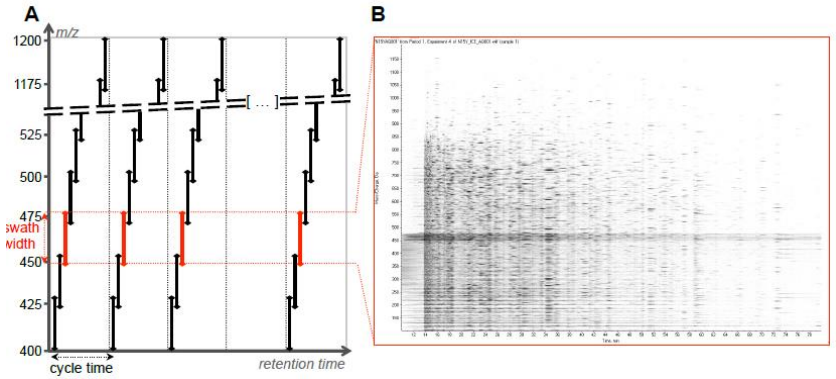
~50 ug peptides from
1mg mouse liver or
human kidney tissue

→

0.3~0.5ug peptides
sufficient for a
swath analysis

6~8 hrs to convert 6 pieces of tissues into clean peptides that are ready for MS analysis

Proteomic analysis using SWATH-MS



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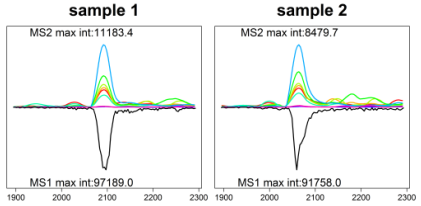
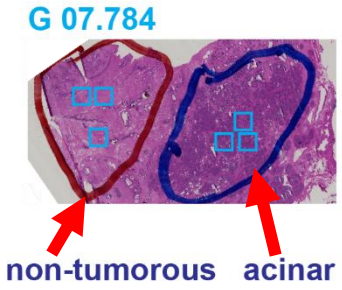


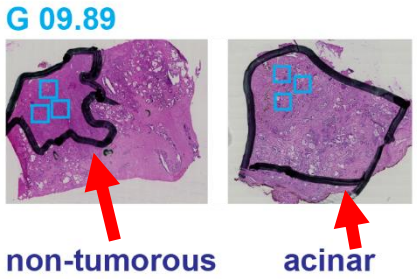
targeted data extaction

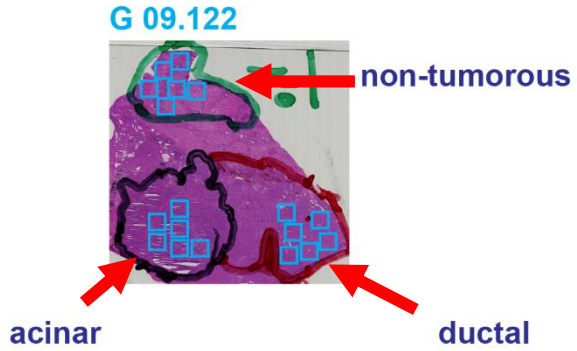
<12hr

Gillet, et al. 2012.

Experimental design







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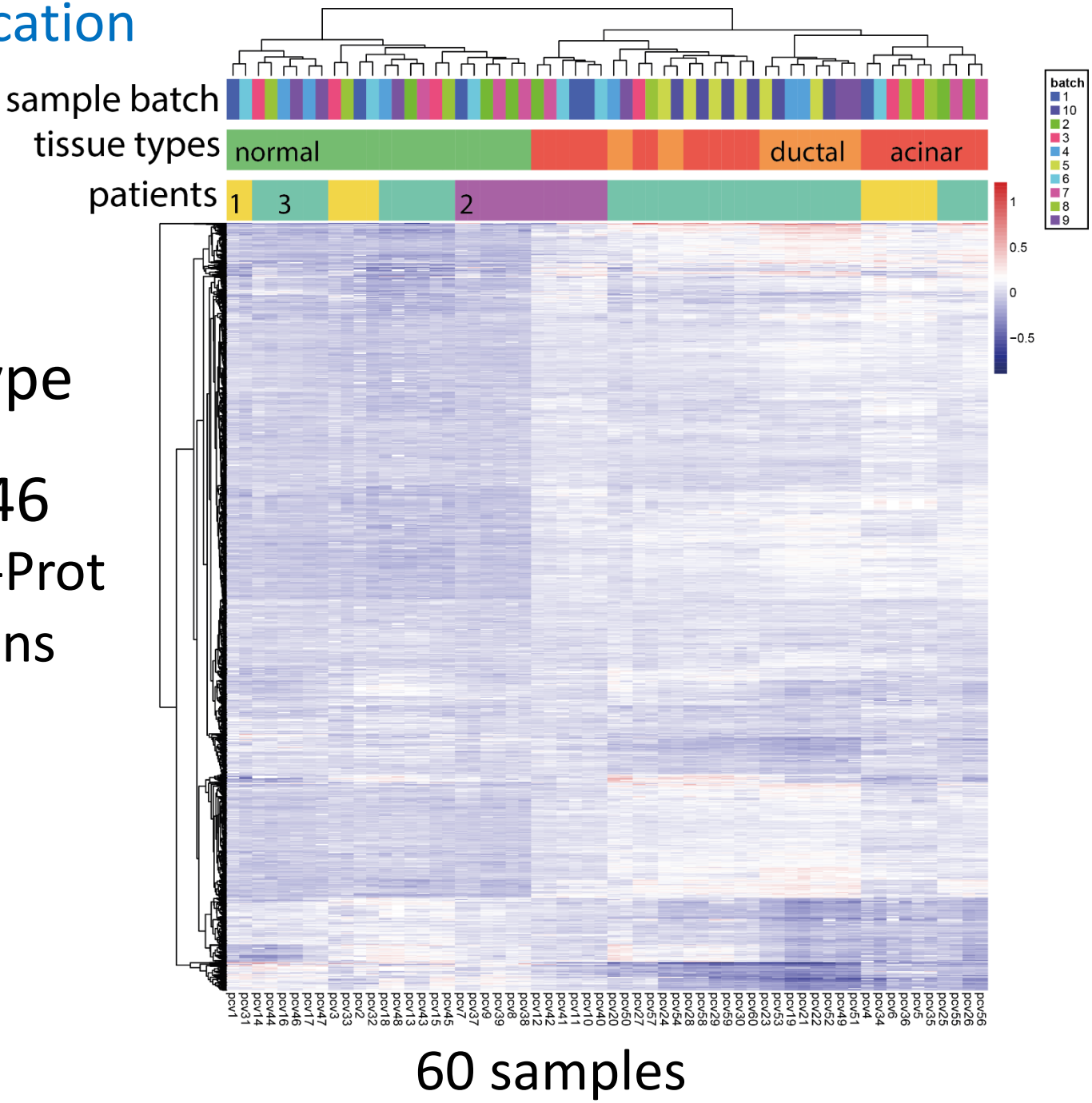
27			3
28			4
29			5
30			6

x2 =60 SWATH runs finished in **9 days** (2hr gradient)

PCT-Sample preparation: 1 + 5 days

Sample	Batches									
	1	2	3	4	5	6	7	8	9	10
1	P1N1a	P2N2a	P1N3a	P3N4a	P3D4a	P1N1b	P2N2b	P1N3b	P3N4b	P3D4b
2	P1N2a	P2N3a	P1A2a	P3N5a	P3D5a	P1N2b	P2N3b	P1A2b	P3N5b	P3D5b
3	P1A1a	P2A3a	P1A3a	P3N6a	P3D6a	P1A1b	P2A3b	P1A3b	P3N6b	P3D6b
4	P2A1a	P3A1a	P3N2a	P3D1a	P3A4a	P2A1b	P3A1b	P3N2b	P3D1b	P3A4b
5	P2A2a	P3A2a	P3N3a	P3D2a	P3A5a	P2A2b	P3A2b	P3N3b	P3D2b	P3A5b
6	P2N1a	P3N1a	P3A3a	P3D3a	P3A6a	P2N1b	P3N1b	P3A3b	P3D3b	P3A6b

Protein quantification



Proteotype

1946
Swiss-Prot
proteins

60 samples

Conclusions

- 1, PCT-SWATH permits the first investigation of intra-tumor heterogeneity at proteomic level
- 2, We observed both homogeneity and heterogeneity at proteomic level.
- 3, Proteomic features are reasonable reflection of the phenotypes. They will find applications in personalized/precision medicine.

Acknowledgements

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AB SCIEX