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Introducing Tissue Microarrays to Molecular Pathology

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Featured Article: Tissue microarrays for high-throughput molecular profiling of tumor specimens. Kononen J, Bubendorf L, Kallioniemi A, Bärlund M, Schraml P, Leighton S, Torhorst J, Mihatsch MJ, Sauter G, Kallioniemi OP. Nat Med. 1998;4:844–7.[2]

Tissue specimens are essential for biomedical and clinical research. In situ detection of DNA, RNA, and protein targets in tissues provides a powerful means to understand disease at the level of the tissue microenvironment and to develop diagnostic biomarkers. However, biobanked samples are often small and valuable collections are depleted over time. The analysis of tissue biomarkers is also labor intensive, slow, and expensive. In 1997, with these limitations in mind, we started to develop array-based technologies for high-throughput in situ analysis of biobanked tissues and tumors.

In tissue microarray (TMA) technology, tissue cores from hundreds to up to 1000 tissues are brought together in a regular array format, sectioned, and applied for molecular analyses on microscope slides *(1)*. Hundreds of consecutive slides can be analyzed with different antibodies and probes, thus facilitating the rapid analysis of many tissues at a time and also of many biomarkers/targets from consecutive sections.

Interestingly, what is now considered a routine research tool in molecular pathology began its life in the Cancer Genetics Branch of the National Human Genome Research Institute at NIH. This happened at a time when technologies such as DNA microarrays had just started to transform genomics research. In that environment, it was natural for us to consider applying high-throughput, highly parallel, automated approaches to molecular pathology. We teamed up with an engineer, Steve Leighton, and built the first manual tissue arrayer prototype. Guido Sauter provided feedback from the pathology laboratory as well as ideas and enthusiasm regarding the application of the technology to tissue banks composed of tens of thousands of samples, as subsequently documented in many joint publications that were collaborations between investigators at NIH and in Basel *(2, 3)*.

We decided to use cores of 0.6 μm in diameter from each of the tissues *(1)*. This allowed us to squeeze almost 1000 samples on a single microscope slide, which was almost an order of magnitude more than with previous "multitissue block"–based strategies *(4)*. At the same time, the small size of the tissue sample became the target of criticism: how can such a small piece be representative of a heterogeneous tumor? Even today, this question is discussed in TMA publications *(5)*. Obviously, TMAs are optimized for throughput and one cannot at the same time provide a thorough investigation of each sample. TMAs are typically applied to investigate entire biobanks or cohorts of samples, not individual tumors. TMAs provide an estimate of biomarker prevalence in the population, but to characterize expression patterns across an entire sample it is necessary to use multiple punches per tumor and use whole tissue sections. From genome sequencing we are now learning how multiple tumor subclones can coexist and clonally evolve in different parts of the tumor. Thus, no single piece of the tumor, large or small, would ever fully account for all the various cancer cell clones and subclones in each patient.

Where has the TMA technology taken us in the past 15 years, and what will the future look like? Molecular pathology and in situ detection of genomic alterations and protein targets are as important, if not more important, today as in 1998. TMAs are commonly applied by cancer researchers, molecular pathologists, biomedical and clinical research groups, hospitals, and developers of diagnostic assays. Large-scale resources of immunostained tissues and tumors profiled on TMAs are now available in public web pages *(6)*, such as the Human Protein Atlas (http://www.proteinatlas.org/). The importance of such reference data sources is bound to increase and is promoted by innovations in digital pathology. When linked to clinical and follow-up patient data, physical and digital TMA repositories will facilitate discovery of patient subgroups for therapy and companion diagnostics. We will need to better integrate the exponentially increasing cancer (gen)omics data with the phenotypes of the tumor and the microenvironmental context. The availability of tissue biobanks in TMA format still needs

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[2] This article has been cited more than 2195 times since its publication.

improvement, particularly from clinical trials. We predict that TMAs will continue to be key tools for biomedical, translational, and clinical researchers as well as for diagnostics and personalized medicine, at least for the next 15 years.

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References

1. Kononen J, Bubendorf L, Kallioniemi A, Bärlund M, Schraml P, Leighton S, et al. Tissue microarrays for high-throughput molecular profiling of tumor specimens. Nat Med 1998;4:844–7.
2. Schraml P, Kononen J, Bubendorf L, Moch H, Bissig H, Nocito A, et al. Tissue microarrays for gene amplification surveys in many different tumor types. Clin Cancer Res 1999;5:1966–75.
3. Kallioniemi OP, Wagner U, Kononen J, Sauter G. Tissue microarray technology for high-throughput molecular profiling of cancer. Hum Mol Genet 2001;10:657–62.
4. Battifora H, Mehta P. The checkerboard tissue block: an improved multitissue control block. Lab Invest 1990;63:722–4.
5. Seol H, Lee HJ, Choi Y, Lee HE, Kim YJ, Kim JH, et al. Intratumoral heterogeneity of HER2 gene amplification in breast cancer: its clinicopathological significance. Mod Pathol 2012;25:938–48.
6. Uhlen M, Oksvold P, Fagerberg L, Lundberg E, Jonasson K, Forsberg M, et al. Towards a knowledge-based Human Protein Atlas. Nat Biotechnol. 2010;28:1248–50.