

Meeting report  
**Probing the cancer genome**  
Heidi Greulich

Address: Dana-Farber Cancer Institute, Binney St, Boston, MA 02115, and Broad Institute of MIT and Harvard, Cambridge Center, Cambridge, MA 02142, USA. Email: heidig@broad.mit.edu

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A report on the Keystone Symposium 'Cancer Genomics and Epigenomics', Taos, USA, 19-24 February 2008.

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Unlike heart disease, the overall mortality rate for cancer has not significantly improved over the past 50 years, indicating an unmet need for better cancer treatments, in particular for targeted therapies that are more effective and less toxic than traditional cytotoxic chemotherapy. Development of such therapies requires a detailed understanding of cancer genomes, including the effects of epigenomic modifications. A recent Keystone Meeting on cancer genomics and epigenomics addressed this knowledge gap, and some of the highlights are presented here.

### Genomic lesions in cancer cells

Complete characterization of lesions in the genomes of tumor cells is fast becoming a reality. The extent to which technology drives discovery was especially apparent in the talks on cancer genome analysis. Next-generation single-molecule sequencing technologies, such as those developed by the companies Solexa and 454, have already been successfully applied to answer questions about both genomic and epigenomic organization. In addition, many participants reported the application of more traditional approaches to characterization of the three main types of genomic lesions in cancer: translocations, copy-number changes and point mutations.

High-throughput discovery of translocations has been explored by both array-based and sequencing-based approaches, but has proved to be difficult. Aleks Milosavljevic (Baylor College of Medicine, Houston, USA) has used a combination of traditional Sanger sequencing and 454 single-molecule pyrosequencing to identify translocations in bacterial artificial chromosome (BAC) clones created from the MCF-7 breast cancer cell line. His group successfully identified 157 PCR-confirmed breakpoint junctions, 10 of which were located in introns so that the reading frame was preserved. Several of these in-frame translocations were also confirmed

at the transcript level. It is anticipated that developments in single-molecule sequencing technology will further facilitate this approach.

In contrast to translocation discovery, global assessment of copy-number changes by comparative genomic hybridization (CGH) and its more recent variants is well established. However, increased resolution of segment breakpoints can be theoretically achieved using single-molecule sequencing technologies, and Matthew Meyerson (Dana-Farber Cancer Institute, Boston, USA) presented preliminary data on the development and application of Solexa sequencing for use in higher-resolution digital karyotyping of copy-number alterations.

Copy-number data obtained by these techniques can be used to subclassify tumor samples by patterns of amplification and deletion. These data can also be computationally analyzed to identify putative driver genes under positive selection in recurring copy-number lesions, and many groups are actively working on such algorithms. Michael Wigler (Cold Spring Harbor Laboratory, USA) presented a metric in which each deletion/amplification event is assumed to have a driver, the assigned weight for which is inversely proportional to the number of genes in the DNA segment affected. Meyerson presented the technique of 'genomic identification of significant targets in cancer' (GISTIC), which assigns a score to each amplified or deleted segment on the basis of its frequency and the number of copies deleted or amplified. He reported the application of GISTIC to lung adenocarcinoma copy-number data from the Tumor Sequencing Project, which identified a novel recurrently amplified lineage-specific oncogenic transcription factor in lung adenocarcinoma, *NKX2-1*. Donna Albertson (University of California, San Francisco, USA) and her colleagues have combined copy-number and expression information to identify the gene *GLI2* as the putative driver of a narrow amplicon in oral squamous cell carcinomas. She also reported the experimental investigation of the functional consequences of *GLI2* overexpression by co-culture of HaCat keratinocytes ectopically expressing *GLI2* with fibroblasts in three-dimensional organotypic cultures.

Single-molecule sequencing is also poised to revolutionize mutation discovery in tumor DNA. As the cost decreases, it should soon be possible to routinely sequence all expressed genes in many samples, with the ultimate goal of identifying novel therapeutic targets. I presented data on therapeutic targets that have been identified by old-fashioned Sanger sequencing, including the mutations in the kinase domain of the epidermal growth factor receptor gene (*EGFR*) in lung adenocarcinoma, which predict response to *EGFR*-targeted therapeutics such as erlotinib. More recently, we have identified *EGFR* extracellular domain mutations in glioblastoma, as well as mutations in the fibroblast growth factor receptor 2 gene (*FGFR2*) that confer sensitivity of endometrial carcinoma cell lines to *FGFR* inhibitors.

### Epigenomic changes in cancer

Talks on cancer epigenomics substantially outnumbered those on the genome, indicating the growing interest in this exciting field. Many candidate driver genes have been identified by examination of differentially methylated sequences in tumor cells using methylation-sensitive restriction enzymes. For example, Kornelia Polyak (Dana-Farber Cancer Institute, Boston, USA) has examined genes that are differentially methylated in breast epithelium bipotential progenitor cells compared with a more differentiated luminal epithelial cell population. She found that the transcription factor *FOXC1* is hypomethylated in the progenitor population in both normal and neoplastic breast tissue, and that ectopic expression of *FOXC1* in differentiated mammary epithelial cells induced a progenitor-like migratory and invasive phenotype.

Tomas Ekstrom (Karolinska Institute, Stockholm, Sweden) described a method for detecting the global extent of cytosine methylation - the 'luminometric methylation assay' (LUMA). His group has used this assay to show that infection with human cytomegalovirus (CMV), which is associated with several cancers but is not known to be sufficient for tumor initiation, results in a global reduction of DNA methylation correlated with nuclear exclusion of DNA methyltransferases, raising the possibility of an epigenetic contribution of CMV to cancer.

Single-molecule sequencing has also propelled the study of the cancer epigenome forward. Stephan Beck (University College London, UK) presented data coupling precipitation of methylated DNA sequences by antibody specific for 5-methyl cytosine (meDIP) with Solexa sequencing, showing that this provides increased resolution over the more conventional array-based identification of meDIP-precipitated sequences (meDIP-chip).

Bradley Bernstein (Massachusetts General Hospital, Boston, USA) has combined Solexa sequencing with chromatin immunoprecipitation (ChIP-Seq) in a genome-wide study of the correlation between histone H3 lysine methylation and

transcriptional activity in an effort to better understand the transition from pluripotent stem cell to lineage-committed cell. Sheared chromatin from mouse embryonic stem cells (ES cells), neural progenitor cells (NPCs) and mouse embryo fibroblasts (MEFs) was precipitated with antibodies to specific methylation states of histone H3, and the eluted DNA used to make libraries for Solexa sequencing. The results showed that CpG-rich promoters in ES cells were primarily associated with tri-methylated H3 lysine 4 (H3K4me3), a modification associated with transcribed chromatin, and likely to denote housekeeping genes. However, a small fraction of CpG-rich promoters in the ES cells was also precipitated along with tri-methylated H3 lysine 27 (H3K27me3), and these doubly modified promoters were for the most part not transcriptionally active. Many of the doubly modified promoters in ES cells had only a single modification in the lineage-committed NPCs and MEFs; for example, the promoter of the neural transcription factor gene *OLIG1* was associated with H3K4 tri-methylation in NPCs but only H3K27 tri-methylation in MEFs. Bernstein also showed that retention of H3K27 methylation in differentiated cells is dependent on expression of the Polycomb repressive complex protein PRC1.

The special AT-rich binding protein 1 (*SATB1*) binds base-unpairing regions (BURs) of genomic DNA to organize chromatin into loops. It also establishes region-specific epigenetic status at its target gene loci by recruiting chromatin-remodeling factors. Terumi Kohwi-Shigematsu (Lawrence Berkeley National Laboratory, Berkeley, USA) described the cloning of genomic sequences juxtaposed to the BUR associated with the *MYC* locus. She and her colleagues found that genes encoding known c-Myc binding partners and other signaling proteins controlling *MYC* expression are brought into close proximity to the *MYC* gene in the nuclear space. *SATB1* is overexpressed in metastatic breast cancer cell lines and is associated with poor prognosis in primary breast tumor samples.

### Screening for potential therapeutic targets

One goal of the characterization of genomic and epigenomic lesions in cancer, in addition to a deeper understanding of basic cancer biology, is the identification of novel therapeutic targets. Functional genomics provides an approach to identifying such targets even in the absence of a comprehensive description of the cancer-associated genomic lesions. A number of excellent talks described RNA interference screens using small hairpin RNA (shRNA) to reveal tumor dependencies and genes responsible for drug resistance. René Bernards (Netherlands Cancer Institute, Amsterdam, the Netherlands) described an shRNA screen to identify genes that confer resistance to the antibody trastuzumab (Herceptin) in BT-474 breast cancer cells when knocked down. *PTEN*, a negative regulator of the phosphatidylinositol 3-OH kinase gene *PIK3CA*, was identified in this screen.

Overexpression of oncogenically activated *PIK3CA* was also sufficient to confer resistance to trastuzumab in the SK-BR3 breast cancer cell line. Bernards also reported that breast cancer patients whose tumors were characterized by either low levels of *PTEN* expression or *PIK3CA* mutations (mutually exclusive) exhibited a statistically significant decrease in survival following trastuzumab-based therapy. Consistent with these results, he reported that inhibitors of PI3K overcome trastuzumab resistance due to *PTEN* loss or *PIK3CA* mutation in cultured cells.

Owing to these technological advances, we are now poised for a comprehensive characterization of the cancer genome. There is no doubt that exponentially increasing volumes of data will be generated, necessitating development of computational approaches to make full use of the biologically relevant information contained within.