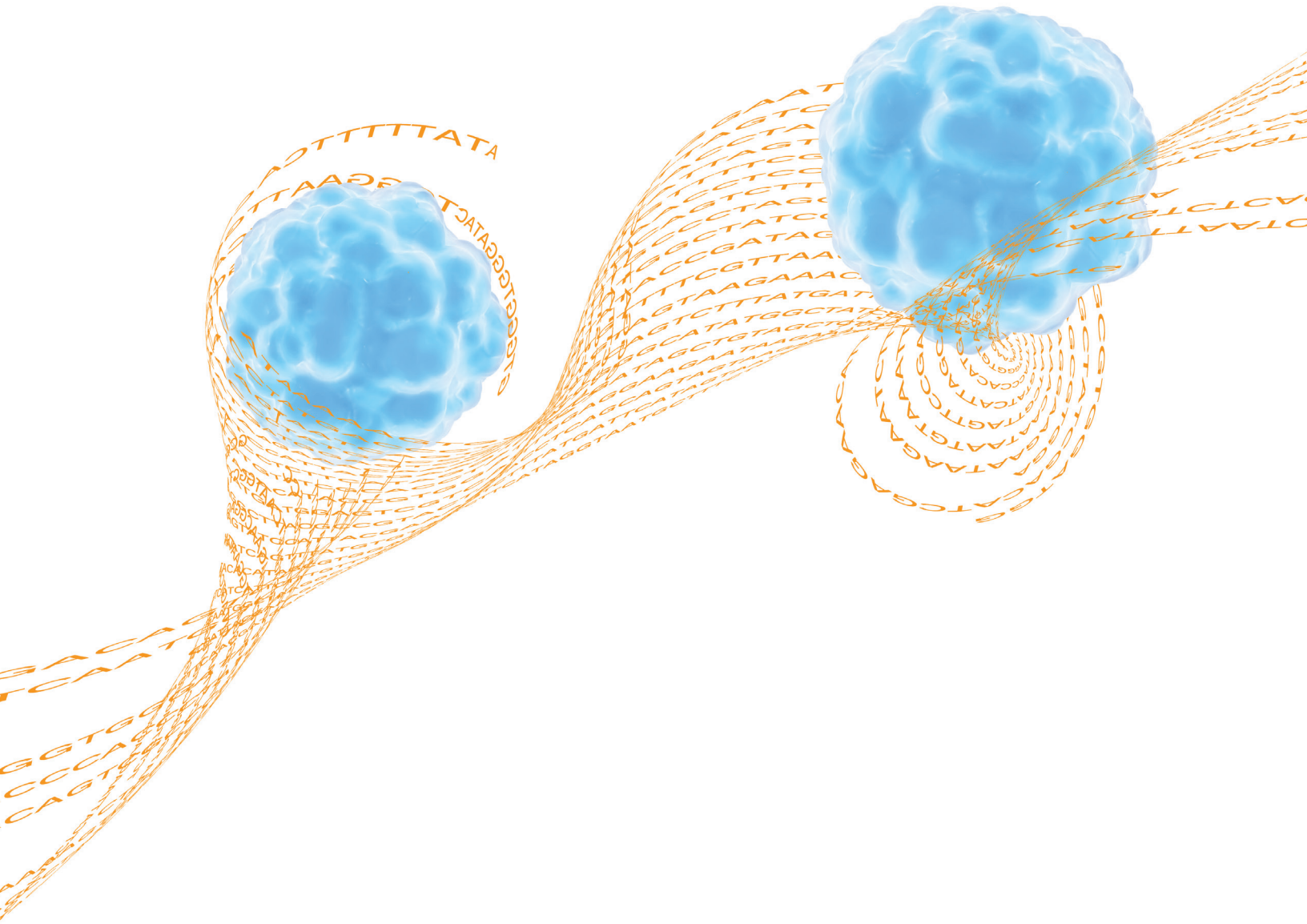
Recent Discoveries in Cancer Genomics

A review of cancer research publications featuring Illumina technology



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Introduction

New sequencing technologies and higher content microarrays are providing unprecedented opportunities for rapidly and cost-effectively monitoring and exploring the genome. These powerful tools are transforming the field of cancer research. The data surge has only just begun, and future studies will inevitably include larger numbers of patients, more detailed analyses, and the discovery of additional molecular phenotypes. Soon, we can expect to see comprehensive catalogs of alterations within cancer genomes, providing an integrated view of all the processes impacted by cancer genome dynamics. Ultimately, this research may lead to the development of better patient diagnoses, therapies, and treatments.

This document highlights recent cancer research studies enabled by advanced Illumina sequencing and microarray technologies. To learn more about the platforms and assays cited, visit www.illumina.com.

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Cancer Biology

Tumor Progression

Understanding tumor progression is critical for effective cancer treatment. Patients are usually screened at a single time point from which the stage of progression is inferred and the best treatment determined. To develop effective anti-cancer drugs, we need to understand the mechanism and pathways involved in tumor progression.

Cancer cell genomes carry somatic mutations. These may include base substitutions, small insertions and deletions (indels), rearrangements, and copy number alterations. As the tumor progresses, somatic mutations accumulate and leave characteristic genomic trails. Sequencing allows us to read these trails with great clarity and resolution. To interpret them, we need a comprehensive reference set of mutations for different tumor types. This will allow us to relate the mutations back to disease mechanisms and progression to better inform diagnosis and treatment.

A comprehensive catalogue of somatic mutations from a human cancer genome

Pleasance ED, Cheetham RK, Stephens PJ, McBride DJ, Humphray SJ, et al. (2010) Nature 463: 191–6.

This paper presents a catalog of somatic mutations from the genomes of a malignant melanoma and COLO-829, a lymphoblastoid cell line from the same person in an effort to understand DNA damage, mutation, repair, and selection in a cancer genome. The mutations in COLO-829 exhibit evidence of past ultraviolet light–induced DNA damage and auxiliary, independent mechanisms of damage. There are also traces of DNA repair processes, including transcription-coupled nucleotide excision repair (NER) and other, less well characterized patterns of NER deployment. Generating catalogs such as this will help increase our understanding of cancer causation and development, providing the foundation for prevention and treatment.

Illumina technology: Genome Analyzer$_{II}$

Tracing the tumor lineage

Navin NE and J Hicks (2010) Mol Oncol 4: 267–83.

In an effort to understand the fundamental basis of tumor heterogeneity and progression through single cell genomics, the authors developed a single nucleus sequencing (SNS) method. This method aligns a massive number of sequencing reads to the human genome and measures read depth in 50-kb fixed intervals, resulting in a copy number profile. Profiles from the same tumor often clustered into a few highly similar groups, rather than a series of gradual intermediates or unrelated profiles, consistent with the clonal expansion model. The single cell analysis approach adds an additional dimension to our understanding of tumor progression and opens up new avenues for research and treatment.

Illumina technology: Genome Analyzer$_{II}$

Malignant precursor cells pre-exist in human breast DCIS and require autophagy for survival

Espina V, Mariani BD, Gallagher RI, Tran K, Banks S, et al. (2010) PLoS ONE 5: e10240.

Fresh human ductal carcinoma in situ (DCIS) lesions contain pre-existing carcinoma precursor cells. To identify and characterize the tumorigenic cells within the DCIS tissue responsible for the phenotype, the authors cultured fresh living human DCIS ductal fragments to generate DCIS neoplastic cell outgrowths. The outgrowths spontaneously generated 3D spheroids and duct-like structures. The results indicate that autophagy is required for survival and anchorage-independent growth of the cytogenetically abnormal tumorigenic DCIS cells.

Illumina technology: Infinium® HumanCytoSNP-12 BeadChip

Angiogenesis

Angiogenesis is the natural process of blood vessel growth that requires a careful balance between stimulation and inhibition. This process is co-opted during tumor development to generate a network of blood vessels that penetrates into cancerous growths to supply nutrients and oxygen. Blood vessels within tumors are usually formed by sprouting of resident tissue endothelial cells. This process is critical for tumor growth and metastasis.

Elevated AKR1C3 expression promotes prostate cancer cell survival and prostate cell-mediated endothelial cell tube formation: implications for prostate cancer progress

Dozmorov MG, Azzarello JT, Wren JD, Fung KM, Yang Q, et al. (2010) BMC Cancer 10: 672.

In localized and advanced prostate adenocarcinoma, the aldo-keto reductase (AKR) 1C family member 3 (AKR1C3), is usually up-regulated. This process is associated with prostate cancer (PCa) aggressiveness. Microarray analysis of a stably AKR1C3-transformed PC-3 prostate cancer cell line reveals that AKR1C3 overexpression promotes angiogenesis and growth of PC-3 cells. These results suggest that AKR1C3-mediated tumor angiogenesis is regulated by estrogen and androgen metabolism with subsequent IGF-1R and Akt activation followed by VEGF expression.

Illumina technology: HumanWG-6 v2.0 Expression BeadChip (discontinued product)

Additional References

- The miR-15/107 group of microRNA genes: Evolutionary biology, cellular functions, and roles in human diseases
  Finnerty JR, Wang WX, Hebert SS, Wilfred BR, Mao G, et al. (2010) J Mol Biol 402: 491–509.
- Inhibition of neovascularization to simultaneously ameliorate graft-vs-host disease and decrease tumor growth
  Penack O, Henke E, Suh D, King CG, Smith OM, et al. (2010) J Natl Cancer Inst 102: 894–908.

Metastasis

Metastasis is a complex process by which cancer cells break away from the primary tumor and circulate through the bloodstream or lymphatic system to other sites in the body. At the new sites, the cells continue to multiply and eventually form additional tumors. The ability of pancreatic cancer and uveal melanomas to metastasize contributes greatly to their lethality. Many fundamental questions remain about the clonal structures of metastatic tumors, phylogenetic relationships among metastases, the scale of ongoing parallel evolution in metastatic and primary sites, and how the tumor disseminates.

The patterns and dynamics of genomic instability in metastatic pancreatic cancer

Campbell PJ, Yachida S, Mudie LJ, Stephens PJ, Pleasance ED, et al. (2010) Nature 467: 1109–13.

Pancreatic cancer has a distinctive pattern of genomic instability, dominated by breakage-fusion-bridge. This paper annotates genomic rearrangements in 13 patients with pancreatic cancer and explores the resulting clonal relationships.

Illumina technology: Genome Analyzer$_{II}$

The power of NGS technologies to delineate the genome organization in cancer: from mutations to structural variations and epigenetic alterations

Schweiger MR, Kerick M, Timmermann B, and M Isau (2011) Cancer Metastasis Rev [ePub ahead of print].

The authors review the current status of integrative cancer genomic approaches and the use of massively parallel sequencing in oncology, providing a future perspective of both clinical and basic research. They genotyped 206 rearrangements across multiple lesions from ten patients. Many rearrangements occurred in the primary tumor before metastasis commenced, and were present in all metastases. However, in several patients there was evidence for ongoing clonal evolution in the primary tumor among cells capable of initiating metastases, while other patients showed signs of clonal evolution within the metastasis. This observation provides evidence of organ-specific mutations in the metastases.

There are two explanations for organ-specific mutations. First, particular genotypes might drive metastasis to particular organs. The lung metastases in two patients were associated with additional driver mutations (amplification of MYC or CCNE1), indicating that tumor cells from subclones carrying these rearrangements were more likely to survive in the lung. Second, metastatic spread may be a stepwise process that occurs more readily within organ boundaries than between organs. These explanations are not mutually exclusive. To overcome the barrier to colonization, a subclone of cancer cells may acquire particular adaptive changes in order to disseminate through the organ.

Illumina technology: Genome Analyzer$_{II}$

Genome remodelling in a basal-like breast cancer metastasis and xenograft

Ding L, Ellis MJ, Li S, Larson DE, Chen K, et al. (2010) Nature 464: 999–1005.

The authors analyzed DNA samples from peripheral blood, the primary tumor, a brain metastasis, and a xenograft derived from the primary tumor of an African-American patient with basal-like breast cancer. The metastasis contained two de novo mutations, a large deletion not present in the primary tumor, and was significantly enriched for 20 shared mutations. The xenograft retained all primary tumor mutations and displayed a mutation enrichment pattern that resembled the metastasis. The differential mutation frequencies and structural variation patterns in metastasis and xenograft compared with the primary tumor indicate that secondary tumors may arise from a minority of cells within the primary tumor.

Illumina technology: Genome Analyzer

Frequent mutation of BAP1 in metastasizing uveal melanomas

Harbour JW, Onken MD, Roberson ED, Duan S, Cao L, et al. (2010) Science 330: 1410–3.

Through sequencing, inactivating somatic mutations were identified in the gene encoding BRCA1-associated protein 1 (BAP1) on chromosome 3p21.1 in 26 of 31 (84%) metastasizing tumors, implicating loss of BAP1 in uveal melanoma metastasis. This suggests that the BAP1 pathway may be a potential therapeutic target.

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Cancer Mechanisms

The advent of massively parallel sequencing provides an unprecedented toolbox to untangle the causes and mechanisms of cancer.

Gene Fusions

Gene fusions are hallmarks of some cancer types, formed by the fusion of two previously separate genes. Fusions may lead to a gene product with a new or different function from the two fusion partners. The combination of a strong promoter with a functional gene (proto-oncogene) downstream is common in some cancers. The mechanisms of creating fusion genes are as varied as the functions of the resultant genes. There are several approaches to studying fusion genes through sequencing, such as whole-genome sequencing of the tumor, exome sequencing, and mRNA-Seq.

Whole-Genome Sequencing to Detect Gene Fusions

Sequencing the whole genome is a rigorous approach to finding all variants. Provided the coverage is deep enough, the investigator can be sure no mutation will go undetected and valuable samples will not have to be resequenced in the future. In the following examples, some of the gene fusions may have been missed with more targeted approaches such as exome sequencing or microarrays. Sequencing the whole genome allows the integration with ChIP-Seq data, significantly expanding the data interpretation.

The genomic complexity of primary human prostate cancer

Berger MF, Lawrence MS, Demichelis F, Drier Y, Cibulskis K, et al. (2011) Nature 470: 214–20.

This paper presents the complete sequence of seven primary human prostate cancers and their paired normal counterparts. Several tumors contained complex chains of balanced (copy-neutral) rearrangements that occurred within, or adjacent to, known cancer genes. Some of the breakpoints occurred in intergenic regions that may have been missed by exon-targeted approaches. In 88% of the cases, the fusion point could be mapped to base pair resolution. The most common type of fusion involved a precise join, with neither overlapping nor intervening sequence at the rearrangement junction. This result differed from the patterns seen in breast tumors, in which the most common junction involved a microhomology of 2–3 bp , indicating that the mechanisms responsible for generating these fusions are different for prostate and breast cancers.

Illumina technology: Genome Analyzer

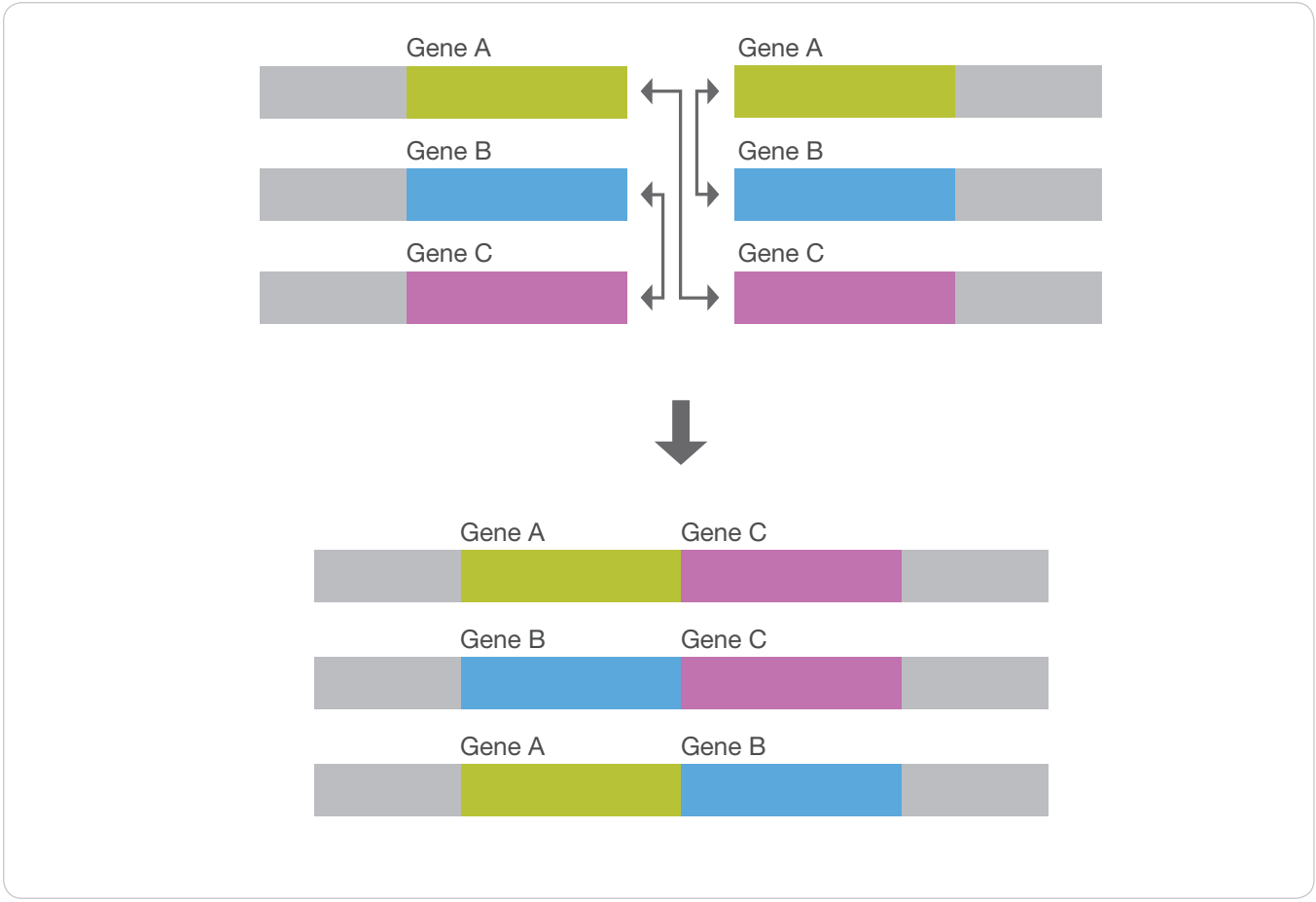

Figure 1. Complex rearrangement in prostate tumor PR-2832 involving breakpoints and fusions at nine distinct genomic loci. Sequences are duplicated or deleted in the derived chromosomes at the resulting fusion junctions. For breakpoints in intergenic regions, the nearest gene in each direction is shown. (Adapted from Berger et al., (2011) Nature 470: 214–20.)

The patterns and dynamics of genomic instability in metastatic pancreatic cancer

Campbell PJ, Yachida S, Mudie LJ, Stephens PJ, Pleasance ED, et al. (2010) Nature 467: 1109–13.

Focusing on the evolution of metastases, the authors found that one-sixth of rearrangements show a distinctive pattern they termed 'fold-back inversions' where a copy number change is demarcated by read-pairs aligning close together but in inverted orientation. As a result, the genomic region is duplicated but the two copies are in opposite orientations next to the breakpoint. The most probable mechanism for this occurrence is thought to be a breakage-fusion-bridge cycle. In this mechanism, a double-stranded DNA break occurring in G0-1 phase is replicated during S phase, leading to two identical DNA ends. The repair pathways directly join these, leading to a fold-back inversion pattern at the junction and an unstable dicentric chromosome.

Illumina technology: Genome Analyzer

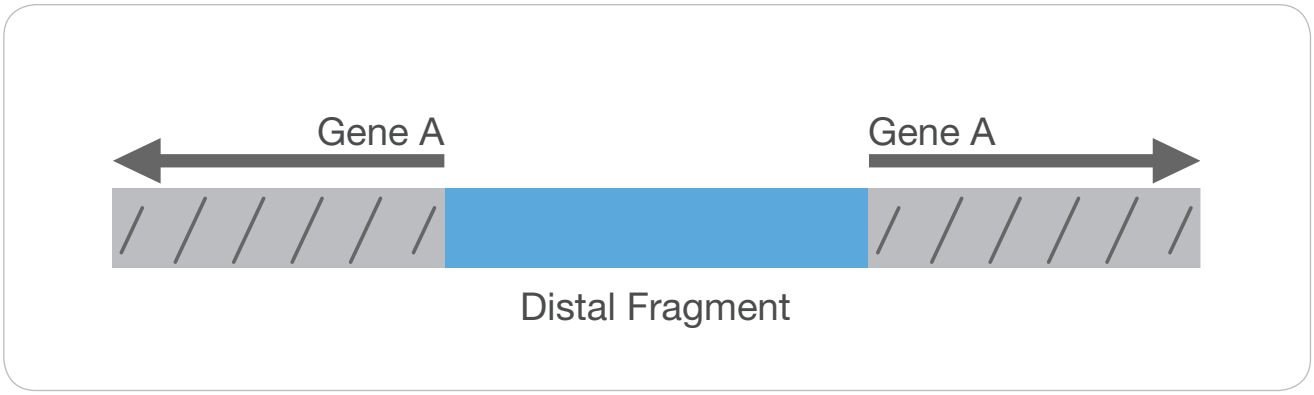

Figure 2. Genomic patterns of fold-back inversions. Some fold-back inversions captured fragments of templated genomic DNA between the two ends. These were often from distant regions of the genome, such as centromeric repeats or adjacent to other dsDNA breaks involved in somatic rearrangements. (Adapted from Campbell et al., (2010) Nature 467: 1109–13.)

Massive genomic rearrangement acquired in a single catastrophic event during cancer development

Stephens PJ, Greenman CD, Fu B, Yang F, Bignell GR, et al. (2011) Cell 144: 27–40.

This paper describes a phenomenon, called chromothripsis, during which tens to hundreds of genomic rearrangements occur in a one-off cellular crisis. This model of a single catastrophic event during cellular duplications is different from the typical model of cancer progression through the progressive accumulation of mutations. The consequence of the catastrophic rearrangements is local regionalization of complex rearrangements and copy number variants with a limited range, 0 –2, indicating that this is the result of a single event. In a cancer progression model where mutations accumulate, there is no upper limit to the copy numbers and it is common to see a wide range of copy numbers. The authors found evidence of chromothripsis in 2–3% of all cancers, across many subtypes, and in ~25% of bone cancers.

Illumina technology: Genome Analyzer$_{II}$

Additional Reference

- Complex landscapes of somatic rearrangement in human breast cancer genomes
  Stephens PJ, McBride DJ, Lin ML, Varela I, Pleasance ED, et al. (2009) Nature 462: 1005–10.

RNA-Seq to Detect Gene Fusions

Using RNA-Seq to detect gene fusions offers distinct advantages. When compared to whole-genome sequencing, much less sequence needs to be generated. When compared to exome sequencing, it avoids the extra exome enrichment sample preparation step. Only known exomes that can be targeted with primers can be enriched, so ***de novo*** exomes that result from fusions may be difficult to interpret or missed. The fundamental compromise inherent in this approach is that only gene fusions expressed at the time of sampling can be detected. This is particularly important for cancers such as breast cancer that respond to hormonal stimulation. In studies such as drug target discovery where we assume that only expressed genes will have an impact on the outcome of the disease, this can be cost-effective approach to screening large numbers of patients.

Discovery of non-ETS gene fusions in human prostate cancer using next-generation RNA sequencing

Pflueger D, Terry S, Sboner A, Habegger L, Esgueva R, et al. (2011) Genome Res 21: 56–67.

The ETS (E-twenty six) family is one of the largest families of transcription factors. ETS fusions are found in more than half of all prostate cancers. However, ETS fusions alone are not enough to form fully-developed cancer in vivo, suggesting that additional processes are involved. The authors used RNA-Seq to survey the whole transcriptome of 25 human prostate cancer samples for the presence of non-ETS fusion transcripts. They also developed a program, FusionSeq, in order to identify novel gene fusion candidates with high confidence. This resulted in the discovery and characterization of seven new cancer-specific gene fusions, two involving the ETS genes ETV1 and ERG, and four involving non-ETS genes such as CDKN1A (p21), CD9, and IKBKB (IKK-beta). The non-ETS fusions were all present in prostate cancer harboring the TMPRSS2–ERG gene fusion.

Illumina technology: Genome Analyzer$_{II}$

Additional References

- Transcriptome sequencing to detect gene fusions in cancer
  Maher CA, Kumar-Sinha C, Cao X, Kalyana-Sundaram S, Han B, et al. (2009) Nature 458(7234): 97–101.
- Chimeric transcript discovery by paired-end transcriptome sequencing
  Maher CA, Palanisamy N, Brenner JC, Cao X, Kalyana-Sundaram S, et al. (2009) Proc Natl Acad Sci U S A 106(30): 12353–12358.

Targeting Gene Fusions in Pre-Clinical Research

Rearrangements of the RAF kinase pathway in prostate cancer, gastric cancer and melanoma

Palanisamy N, Ateeq B, Kalyana-Sundaram S, Pflueger D, Ramnarayanan K, et al. (2010) Nat Med 16: 793–8.

Gene fusions are commonly considered ‘undruggable’ by conventional approaches. Recently, rare targetable gene fusions involving the anaplastic lymphoma receptor tyrosine kinase (ALK) gene have been identified in 1–5% of lung cancers, suggesting that similar rare gene fusions may occur in other common epithelial cancers, including prostate cancer. The authors screened ETS rearrangement-negative prostate cancers for targetable gene fusions and identified the SLC45A3-BRAF (solute carrier family 45, member 3-v-raf murine sarcoma viral oncogene homolog B1) and ESRP1-RAF1 (epithelial splicing regulatory protein-1-v-raf-1 murine leukemia viral oncogene homolog-1) gene fusions. Fusion gene expression in prostate cells induced a neoplastic phenotype sensitive to RAF and mitogen-activated protein kinase (MAP2K1) inhibitors. Recurrent rearrangements in the RAF pathway tend to occur in advanced prostate cancers, gastric cancers and melanoma. This suggests that RAF and MEK inhibitors may be useful in a subset of gene fusion-harboring solid tumors. It also demonstrates that sequencing of tumor transcriptomes and genomes may lead to the identification of rare targetable fusions across cancer types.

Illumina technology: Genome Analyzer$_{II}$

Understanding the Functional Impact of Gene Fusions with ChIP-Seq

An integrated network of androgen receptor, polycomb, and TMPRSS2-ERG gene fusions in prostate cancer progression

Yu J, Mani RS, Cao Q, Brenner CJ, Cao X, et al. (2010) Cancer Cell 17: 443–54.

Gene fusions of TMPRSS2 to the oncogenic ETS transcription factor ERG occur in approximately 50% of prostate cancers. To understand the functional impact of this fusion product, the authors used ChIP-Seq of AR (androgen receptor), ERG and a combination of histone markers. The ChIP process was carried out in two stages. ChIP was first performed using an anti-ERG antibody and the second ChIP was performed using an anti-AR antibody. In LNCaP cells, the study revealed 37,193 AR binding peaks. The authors find that ERG disrupts AR signaling by inhibiting AR expression, binding to and inhibiting AR activity at gene-specific loci, and inducing repressive epigenetic programs via direct activation of the H3K27 ethyltransferase EZH2, a Polycomb group protein. These findings provide a working model in which TMPRSS2-ERG plays a critical role in cancer progression by disrupting lineage-specific differentiation of the prostate and potentiating the EZH2-mediated dedifferentiation program.

Illumina technology: Genome Analyzer

Additional Reference

- Constitutional translocation breakpoint mapping by genome-wide paired-end sequencing identifies HACE1 as a putative Wilms tumour susceptibility gene
  Slade I, Stephens P, Douglas J, Barker K, Stebbings L, et al. (2010) J Med Genet 47(5): 342–347.

Aneuploidy

Aneuploidy is defined as an abnormal number of chromosomes that arise from improper chromosomal separation during cell division and are a well-established cause of tumor development.

Identification of aneuploidy-tolerating mutations

Torres EM, Dephoure N, Panneerselvam A, Tucker CM, Whittaker CA, et al. (2010) Cell 143: 71–83.

Aneuploidy causes a proliferative disadvantage in all normal cells, yet cancer is characterized by unabated proliferative potential. The authors use a yeast model to study the mechanisms that allow cancer cells to tolerate the adverse effects of aneuploidy. Results demonstrate the existence of aneuploidy-tolerating mutations that improve the fitness of multiple different aneuploidies and highlight the importance of ubiquitin-proteasomal degradation in suppressing the adverse effects of aneuploidy.

Illumina technology: Genomic DNA Sample Preparation Kit

Reviews

- Cell biology: Aneuploidy and cancer
  D Pellman (2007) Nature 446: 38–39.

Gene Expression

Gene expression analysis provides insight into the molecular functions within the cell. Microarray-based mRNA analysis has been used extensively to study gene expression in cancer research. The advent of sequencing-based mRNA analysis (mRNA-Seq) represents a quantum leap forward in our ability to measure and interpret the products of gene expression.

Integrative analysis of the melanoma transcriptome

Berger MF, Levin JZ, Vijayendran K, Sivachenko A, Adiconis X, et al. (2010) Genome Res 20: 413–27.

Integration of transcriptomic and structural genomic data was used to characterize the spectrum of cancer-associated mRNA alterations. With this integrated approach,11 novel melanoma gene fusions produced by underlying genomic rearrangements, as well as 12 novel readthrough transcripts were identified. The study also revealed a high rate of somatic mutations of 1.1 X 10^{-5}, supporting the hypothesis that point mutation is the major driver of melanoma progression.

Illumina technology: Paired-end RNA-Seq on the Genome Analyzer$_{II}$

Deep RNA sequencing analysis of readthrough gene fusions in human prostate adenocarcinoma and reference samples

Nacu S, Yuan W, Kan Z, Bhatt D, Rivers CS, et al. (2011) BMC Medical Genomics 4: 11.

To search for transcription-induced chimeras (TICs) the authors performed single-end RNA-Seq on three human prostate adenocarcinoma samples and corresponding normal tissues, as well as brain and universal reference samples. Their targeted alignment strategy allowed them to effectively identify TIC events across the genome in individual tissues. Prostate and reference samples exhibit a wide range of TIC events, involving more genes than estimated previously using ESTs. This is may be due to the increased sensitivity of the sequencing approach.

Illumina technology: RNA-Seq on the Genome Analyzer

Reviews

- Alternative expression analysis by RNA sequencing
  Griffith M, Griffith OL, Mwenifumbo J, Goya R, Morrisy JS et al. (2010) Nat Methods 7(10): 843–847.
- RNA-Seq: A revolutionary tool for transcriptomics
  Wang Z, Gerstein M and M Snyder (2009) Nat Rev Genet 10: 57–63.

MicroRNA (miRNA)

Small non-coding RNAs called microRNA (miRNA) were discovered in the early 1990s, but were not recognized as a distinct class of biologic regulators with conserved functions until the early 2000s. Using next-generation sequencing, miRNAs can be identified and distinguished with single-base resolution. Mature miRNAs function to regulate gene expression by translational repression or enhanced mRNA degradation.

The study of miRNAs in cancer research falls into two categories. The first is the discovery and functional annotation of miRNAs that play a role in cancer progression. The second is the potential use of miRNAs as markers for the detection and staging of cancer to improve diagnosis and treatment.

Discovery and Functional Annotation of miRNAs in Cancer

Identification of new microRNAs in paired normal and tumor breast tissue suggests a dual role for the ERBB2/Her2 gene

Persson H, Kvist A, Rego N, Staaf J, Vallon-Christersson J, et al. (2011) Cancer Res 71: 78–86.

This paper describes a comprehensive screen of miRNAs associated with breast cancer. The sequencing depth led to the discovery of many new low-expressed miRNAs, highlighting the complexity of small RNA expression in breast cancer. Results revealed 361 new, well-supported miRNA precursors. Nearly two-thirds of these new genes were detected in other human tissues, implying that not only the presence of miRNAs, but the miRNAs expression level is functionally important. A new miRNA, miR-4728, is encoded within the ERBB2/Her2 gene, which is frequently amplified and associated with more aggressive disease and shorter patient survival. It is also significantly over-expressed in tumors and cell lines with ERBB2 amplification.

Illumina technology: Genome Analyzer

Integrated profiling of microRNAs and mRNAs: microRNAs located on Xq27.3 associate with clear cell renal cell carcinoma

Zhou L, Chen J, Li Z, Li X, Hu X, et al. (2010) PLoS ONE 5: e15224.

Using clear cell renal cell carcinoma (ccRCC) samples from 10 patients, the authors found 404 miRNAs and 9,799 mRNAs that were differentially expressed, as well as 56 novel miRNA candidates in at least two samples. In a subsequent screen of 50 ccRCC patients, expression of a miRNA gene containing seven miRNAs of the miR-506 family cluster was down regulated in 76.7% of the cases. This enabled analysis of the interaction between miRNA and mRNA expression levels. Individual pathway enrichment analysis shows that cancer pathways, such as cell cycle, apoptosis, focal adhesion, and ECM receptor interaction play critical roles in ccRCC development.

Illumina technology: Genome Analyzer$_{II}$

Deep sequencing of the small RNA transcriptome of normal and malignant human B cells identifies hundreds of novel microRNAs

Jima DD, Zhang J, Jacobs C, Richards KL, Dunphy CH, et al. (2010) Blood 116: 118–127.

The authors isolated and sequenced total RNA from 31 normal and malignant B cell samples to detect the expression of 333 known miRNAs and 286 candidate novel miRNAs. Of the novel miRNAs, 92% were confirmed with qPCR, suggesting that over a third of the microRNAs within these cell types are currently unknown.

Illumina technology: Genome Analyzer

Characterization of the melanoma miRNAome by deep sequencing

Stark MS, Tyagi S, Nancarrow DJ, Boyle GM, Cook AL, et al. (2010) PLoS ONE 5: e9685.

A comprehensive library of melanoma miRNA sequences from a diverse range of pigment cells, including melanoblasts, melanocytes, congenital nevocytes, acral, mucosal, cutaneous, and uveal melanoma cells was generated. A total of 539 known mature and mature-star sequences involved in melanoma, as well as an additional 279 novel miRNA candidates with potential involvement were identified. The discovery of numerous miRNAs as well as miRNA star sequences highlights the importance of deep sequencing to discover and quantify miRNAs.

Illumina technology: Genome Analyzer$_{II}$

miRNAs as Markers for Cancer Detection and Staging

miRNAs are attractive markers for many diseases due to their ease of measurement, relative stability, and role in controlling large numbers of mRNAs. A long road awaits newly-discovered candidates to become qualified biomarkers, and studies are in the very early stages of discovery. The following papers showcase the progress in this rapidly growing field.

A five-microRNA signature identified from genome-wide serum microRNA expression profiling serves as a fingerprint for gastric cancer diagnosis

Liu R, Zhang C, Hu Z, Li G, Wang C, et al. (2010) Eur J Cancer 47: 784–791.

Early detection dramatically increases the prognosis in gastric cancers. This paper explores the possibility of using miRNAs in serum as a noninvasive method to detect gastric cancer. The initial discovery of differentially expressed miRNA was performed on serum samples pooled from 20 non-metastatic, 20 metastatic gastric cancer patients, and 20 controls. The authors found 19 serum miRNAs that were markedly up-regulated in the gastric cancer patients compared to the controls.

Illumina technology: Genome Analyzer

Ultra-high throughput sequencing-based small RNA discovery and discrete statistical biomarker analysis in a collection of cervical tumours and matched controls

Witten D, Tibshirani R, Gu SG, Fire A and WO Lui (2010) BMC Biol 8: 58.

Small RNA libraries were prepared from 29 human cervical tissue tumor-normal pairs. Sequence data revealed a very broad range of expression levels for known miRNAs. Approximately 6% of miRNAs were detected at high sequence counts (> 104), 14% were in the intermediate range (103–104), and the remaining were at low sequence counts (< 100). This method allowed the authors to identify 67 miRNAs that were differentially expressed between the tumor and normal samples at a false discovery rate less than 0.001.

Illumina technology: Genome Analyzer

Reviews

- Serum microRNAs as powerful cancer biomarkers
  Wittmann J and HM Jack (2010) Biochim Biophys Acta 1806(2): 200–207.
- Circulating microRNA in body fluid: a new potential biomarker for cancer diagnosis and prognosis
  Kosaka N, Iguchi H and T Ochiya (2010) Cancer Sci 101(10): 2087–92.

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- MicroRNA in lung cancer
  Lin PY, Yu SL and PC Yang (2010) Br J Cancer 103(8): 1144–8.
- Transcriptome and targetome analysis in MIR155 expressing cells using RNA-Seq
  Xu G, Fewell C, Taylor C, Deng N, Hedges D, et al. (2010) RNA 16(8): 1610–1622.
- Characterization of the small RNA transcriptomes of androgen dependent and independent prostate cancer cell line by deep sequencing
  Xu G, Wu J, Zhou L, Chen B, Sun Z, et al. (2010) PLoS ONE 5(11): e15519.

Epigenetics

Epigenetics refers to heritable changes in gene expression that are not accompanied by changes in DNA sequence. There is increasing evidence that aberrant epigenetic events contribute to diseases such as cancer. Epigenetic control is mediated thorough multiple processes, including DNA methylation, histone modification, and nucleosome remodeling.

Methylation assays can be divided into array- and sequencing-based assays. Sequencing-based analysis can provide highly detailed maps of methylation patterns, while array-based assays provide a cost-effective tool for screening large numbers of samples at lower resolution.

Additional References

- Next generation sequencing based approaches to epigenomics
  Hirst M, and MA Marra, Briefings in Functional Genomics, 2010. 9(5-6): p. 455–465.
- Quantitative comparison of genome-wide DNA methylation mapping technologies
  Bock C, Tomazou EM, Brinkman AB, Muller F, Simmer F, et al. (2010) Nat Biotechnol 28(10): 1106–1114.
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  PW Laird (2010) Nat Rev Genet 11: 191–203.
- The power of NGS technologies to delineate the genome organization in cancer: from mutations to structural variations and epigenetic alterations
  Schweiger MR, Kerick M, Timmermann B and M Isau M (2011) Cancer Metastasis Rev [ePub ahead of print].
- Comparison of sequencing-based methods to profile DNA methylation and identification of monoallelic epigenetic modifications
  Harris RA, Wang T, Coarfa C, Nagarajan RP, Hong C, et al. (2010) Nat Biotechnol 28(10): 1097–1105.
- Epigenomics of human colon cancer
  Carmona F and M Esteller (2010) Mutat Res 693: 53–60.

Sequencing-Based Methylation Assays

The four most frequently used sequencing-based technologies are the bisulfite-based methods (BS-Seq), reduced representation bisulfite sequencing (RRBS), methylated DNA immunoprecipitation sequencing (MeDIP-Seq), and methylated DNA binding domain sequencing (MBD-Seq). BS-Seq is considered the ultimate methylation assay, covering 91.8% of the human methylation sites (CpGs) at single-base resolution. Under similar conditions, MeDIP-Seq covers 53% of the genome at 150-bp resolution.

Integrated analysis of gene expression, CpG island methylation, and gene copy number in breast cancer cells by deep sequencing

Sun Z, Asmann YW, Kalari KR, Bot B, Eckel-Passow JE, et al. (2011) PLoS ONE 6: e17490.

Reduced representation bisulfite sequencing (RRBS) was used to profile the methylation status of 21,570 CpG islands in eight estrogen receptor positive (ER+) and negative (ER-) breast cancer cell lines. This data was correlated with changes in gene expression and gene copy number changes. Gene expression in cell lines was dominated by ER-associated genes. ER+ and ER- cell lines formed two distinct, stable clusters, and 1,873 genes were differentially expressed in the two groups. The authors identified 149 differentially expressed genes

that exhibited differential methylation of one or more CpG islands within 5 kb of the 5' end of the gene. Further analyses revealed a global pattern of differential CpG island methylation contributing to the transcriptome landscape of ER+ and ER- breast cancer cells and tumors. The role of gene amplification/deletion appears to be more modest, although several potentially significant genes may be regulated by copy number aberrations.

Illumina technology: Genome Analyzer

Epigenetic silencing mediated through activated PI3K/AKT signaling in breast cancer

Zuo T, Liu TM, Lan X, Weng YI, Shen R, et al. (2011) Cancer Res 71: 1752–1762.

Trimethylation of histone 3 lysine 27 (H3K27me3) is a critical epigenetic mark for the maintenance of gene silencing. Additional accumulation of DNA methylation in target loci is thought to cooperatively support this epigenetic silencing during tumorigenesis. Activation of PI3K/AKT signaling can be a trigger of this epigenetic processing at many downstream target genes. DNA methylation can be acquired at the same loci in cancer cells, thereby reinforcing permanent repression in those losing the H3K27me3 mark. Combined epigenetic therapies and signaling-targeted treatments synergistically relieve gene silencing and suppress cancer cell growth in vitro and in xenografts.

Illumina technology: Genome Analyzer$_{II}$

Array-Based Methylation Assays

Array-based assays provide a cost-effective tool for screening large numbers of samples at lower resolution.

Identification of a CpG island methylator phenotype that defines a distinct subgroup of glioma

Noushmehr H, Weisenberger DJ, Diefes K, Phillips HS, Pujara K, et al. (2010) Cancer Cell 17: 510–22.

Among 272 glioblastoma tumors, the authors found a distinct subset of samples that display concerted hypermethylation at a large number of loci. This observation indicates the existence of a glioma-CpG island methylator phenotype (G-CIMP). Patients with G-CIMP tumors also have a distinct clinical phenotype: they are younger at the time of diagnosis and experience significantly improved outcomes. Based on these molecular and clinical grounds, G-CIMP appears to be a distinct subset of human gliomas.

Illumina technology: Infinium® HumanMethylation27 BeadChip

Estrogen and progesterone receptor status affect genome-wide DNA methylation profile in breast cancer

Li L, Lee K, Han W, Choi J, Lee J, et al. (2010) Hum Mol Genet 19: 4273–7.

A genome-wide DNA methylation profiling study was used to evaluate whether the DNA methylation state is different in the estrogen receptor (ER) and progesterone receptor (PR) status of breast cancer. Analysis was performed on 12 ER-/PR- and 12 ER+/PR+ breast cancer tissues assaying 27,578 methylation sites in 14,000 genes. Results revealed significantly different methylation in 148 sites, five of which remained significant after multiple testing (best P value = 3.38×10^{-7}). This is the first genome-wide DNA methylation profiling according to the receptor status of breast cancer.

Illumina technology: Infinium® HumanMethylation27 BeadChip

Additional References

- To infinium, and beyond!
  Wright KD and RJ Gilbertson (2010) Cancer Cell 17(5): 419–420.
- Genome-wide DNA methylation analysis of archival formalin-fixed paraffin-embedded tissue using the Illumina Infinium HumanMethylation27 BeadChip
  Thirlwell C, Eymard M, Feber A, Teschendorff A, Pearce K, et al. (2010) Methods 52: 248–54.
- Methylation profiling identifies 2 groups of gliomas according to their tumorigenesis
  Laffaire J, Everhard S, Idbaih A, Criniere E, Marie Y, et al. (2010) Neuro Oncol 13: 84–98.
- Hepatocellular carcinoma displays distinct DNA methylation signatures with potential as clinical predictors
  Hernandez-Vargas H, Lambert PM, Le Calvez-Kelm F, Gouysse G, McKay-Chopin S, et al. (2010) PLoS ONE 5(3): e9749.
- Global analysis of CpG methylation reveals epigenetic control of the radiosensitivity in lung cancer cell lines
  Kim EH, Park AK, Dong SM, Ahn JH and WY Park (2010) Oncogene 29: 4725–4731.

DNA-Protein Interactions (ChIP-Seq)

ChIP-Seq allows researchers to survey DNA-protein binding sites over a complete genome at single-base pair resolution to survey transcription factor binding and chromatin structure. Numerous studies using the technique have been published, making ChIP-Seq a well-established, robust method.

Reviews

- Genomes in three dimensions
  M Baker (2011) Genomics: Nature 470: 289-294.

Transcription Factors and DNA-Protein Interactions

Transcription factor binding to specific DNA target sequences is the fundamental basis of gene regulation networks. Recent studies show that transcription factors vary greatly in their number of genomic binding sites, and that binding events can significantly exceed the number of known or possible direct gene targets. Defining the relationship between transcription factor binding and target regulation across the entire genome is possible with advances in computing and information processing tools for reconstructing and predicting regulatory networks.

Identification of β-catenin binding regions in colon cancer cells using ChIP-Seq

Bottomly D, Kyler SL, McWeeney SK and GS Yochum (2010) Nucleic Acids Res 38: 5735–5745.

The majority of colorectal cancers exhibit mutations in the adenomatous polyposis coli (APC) gene. Cells with mutant APC genes contain elevated levels of the β-catenin transcription coactivator, leading to abnormal expression of genes controlled by β-catenin/T-cell factor 4 (TCF4) complexes. The authors identified 2,168 β-catenin binding regions in HCT116 human colon cancer cells containing core and extended TCF4 motifs and an AP-1 motif.

Illumina technology: Genome Analyzer$_{II}$

Essential role of microphthalmia transcription factor for DNA replication, mitosis and genomic stability in melanoma

Strub T, Giuliano S, Ye T, Bonet C, Keime C, et al. (2011) Oncogene [ePub ahead of print].

The basic helix-loop-helix microphthalmia transcription factor (MITF) is the master regulator determining the identity and properties of the melanocyte lineage. It is regarded as a lineage-specific 'oncogene' with a critical role in the pathogenesis of melanoma. By combining ChIP-Seq and RNA tag sequencing with (siRNA)-mediated MITF knockdown, the authors show that MITF directly regulates a set of genes required for DNA replication, repair, and mitosis.

Illumina technology: Genome Analyzer$_{IIx}$

Reviews

- Genome-wide transcription factor binding: beyond direct target regulation
  Macquarrie KL, Fong AP, Morse RH and SJ Tapscott (2011) Trends in Genetics 27: 141–148.

Additional References

- ChIP-Seq and functional analysis of the SOX2 gene in colorectal cancers
  Fang X, Yu W, Li L, Shao J, Zhao N, et al. (2010) OMICS 14: 369–84.
- Genome-wide assessment of differential roles for p300 and CBP in transcription regulation
  Ramos YF, Hestand MS, Verlaan M, Krabbendam E, Ariyurek Y, et al. (2010) Nucleic Acids Res 38: 5396–5408.

Chromatin Structure and Histone-DNA Interactions

The role of chromatin remodeling in cancer is complex and relatively poorly understood. In some cases, chromatin remodeling genes are implicated as risk factors, while in many cases it is assumed that the chromatin remodeling is a consequence of the rearrangements of the underlying DNA.

Exome sequencing identifies frequent mutation of the SWI/SNF complex gene PBRM1 in renal carcinoma

Varela I, Tarpey P, Raine K, Huang D, Ong CK, et al. (2011) Nature 469: 539–42.

The authors sequenced the protein coding exome in primary clear cell renal carcinoma (ccRCC) cases and identified the SWI/SNF chromatin remodeling complex gene PBRM1. PBRM1 truncating mutations were found in 41% (92/227) of cases.

Illumina technology: Genome Analyzer$_{IIx}$

Imprinting

Genomic imprinting is an epigenetic process involving methylation and histone modifications in order to achieve monoallelic gene expression without altering the genetic sequence. These epigenetic marks are established in the germline and maintained throughout all somatic cells. DNA methylation is a key component of genomic imprinting regulation, and loss of imprinting is one of the most common genetic abnormalities in growth disorders and cancer.

Allele-specific methylation is prevalent and is contributed by CpG-SNPs in the human genome

Shoemaker R, Deng J, Wang W and K Zhang (2010) Genome Res 20: 883–9.

In diploid mammalian genomes, parental alleles can exhibit different methylation patterns (allele-specific DNA methylation, ASM). The authors surveyed ASM and its correlating SNPs across 16 human pluripotent and adult cell lines, observing ASM on 23–37% of heterozygous SNPs in any given cell line. The authors used bisulfite padlock capture and targeted resequencing with an Illumina Genome Analyzer. Distinct types of ASM were identified across many cell types, suggesting a potential role for CpG-SNP in connecting genetic variation with the epigenome.

Illumina technology: Genome Analyzer

Biomarker Discovery

Human cancers are complex diseases characterized by profound cellular and molecular heterogeneity. Tumors with similar clinical and pathological characteristics may have significantly different patient outcomes. Given this complexity, it is not surprising that despite extensive research, relatively few genomic markers have survived validation to be implemented into routine clinical use.

Clinical and pathological characteristics do not represent the molecular phenotype. For example, if a marker discovery study is based on tumor types that appear clinically and pathologically identical, but actually represent a number of different molecular phenotypes, the results will not be reproducible in a different cohort. Cancers accumulate somatic mutations during the course of the disease. Tumor stage should be considered in order to distinguish between risk markers and prognostic markers.

The introduction of new high-throughput genomic technologies has enabled the simultaneous measurement of multiple genomic alterations, revolutionizing the field of genomic marker research in oncology. Microarrays are capable of screening vast numbers of known markers, and sequencing-based studies are revealing the true complexity of disease phenotypes at a molecular level.

Additional Reference

- Genomic markers for decision making: What is preventing us from using markers?
  Coyle VM and PG Johnston (2010) Nat Rev Clin Oncol 7: 90–7.

Risk Markers

The risk of developing cancer is influenced by genetic or environmental components, or a combination of both, often in a complex relationship. Considering the significant genetic disadvantage of a disease such as cancer, it is not surprising that informative genetic markers are rare in the population. Large studies are required to establish a statistical association between a marker and disease.

A sequence variant at 4p16.3 confers susceptibility to urinary bladder cancer

Kiemeney LA, Sulem P, Besenbacher S, Vermeulen SH, Sigurdsson A, et al. (2010) Nat Genet 42: 415–9.

A genome-wide association study (GWAS) was performed in European case-control sample set with a total of 4,739 urinary bladder cancer (UBC) cases and 45,549 controls to test for the presence of the top 20 markers determined in an earlier Icelandic study. The T allele of rs798766 on 4p16.3 was found to associate with UBC (odds ratio = 1.24, $P = 9.9 \times 10^{-12}$). This is a good example of the study sizes needed find statistically significant markers.

Illumina technology: Infinium HumanHap300 BeadChip, Infinium HumanCNV370-Duo BeadChip (discontinued product)

Genetic variation in RPS6KA1, RPS6KA2, RPS6KB1, RPS6KB2, and PDK1 and risk of colon or rectal cancer

Slattery ML, Lundgreen A, Herrick JS and Wolff RK (2010) Mutat Res 706: 13–20.

A candidate gene association study of genes involved in several pathways central to the carcinogenic process was performed. The study included 1,574 colon cancer cases with 1,940 controls and 91 rectal cancer cases with 999 controls. Genetic variations observed in RPS6KA1, RPS6KA2, and PRS6KB2 were associated with increased risk of developing colon cancer, while only genetic variation in RPS6KA2 was associated with altering risk of rectal cancer. These genes also interacted significantly with other genes operating in similar mechanisms, including Akt1, FRAP1, NFκB1, and PIK3CA.

Illumina technology: Custom GoldenGate® Genotyping Assay

A shared susceptibility locus in PLCE1 at 10q23 for gastric adenocarcinoma and esophageal squamous cell carcinoma

Abnet CC, Freedman ND, Hu N, Wang Z, Yu K, et al. (2010) Nat Genet 42: 764–7.

More than 20% of all deaths in the Taihang Mountains of north central China are attributed to gastric cancer and esophageal squamous cell carcinoma (ESCC). A genome-wide association study (GWAS) was performed with 2,240 gastric cancer cases, 2,115 ESCC cases and 3,302 controls. For gastric cancer and ESCC, multiple genome-wide significant variables were independently identified in the 10q23 region, specifically in the PLCE1 gene. When gastric cancers were subdivided into two anatomic sub-sites, association for gastric cardia cancer became stronger: $P = 4.19 \times 10^{-15}$, OR 1.57. The findings suggest a single locus is associated with risk for both cancers.

Illumina technology: Infinium Human660W-Quad BeadChip

Additional References

- A multi-stage genome-wide association study of bladder cancer identifies multiple susceptibility loci
  Rothman N, Garcia-Closas M, Chatterjee N, Malats N, Wu X, Figueroa JD, et al. (2010) Nat Genet 42(11): 978–984.

Prognostic Markers

As cancer progresses, somatic mutations and genomic rearrangements accumulate and profoundly impact patient prognosis. Informative biomarkers are critical for measuring disease progress and response to treatment.

Initial genome sequencing and analysis of multiple myeloma

Chapman MA, Lawrence MS, Keats JJ, Cibulskis K, Sougnez C, et al. (2011) Nature 471: 467–72.

Whole-genome sequencing (WGS) was performed for 23 multiple myeloma patients and whole-exome sequencing (WES; assessing 164,687 exons) was performed for 16 patients, with one patient analyzed by both approaches. Each tumor was compared to its corresponding normal. The frequency of tumor-specific point mutations was 2.9 per million bases, corresponding to approximately 7,450 point mutations per sample across the genome, including an average of 35 amino acid–changing point mutations plus 21 chromosomal rearrangements that disrupted protein-coding regions. Nearly half the patients showed mutations in genes involved in RNA-processing, protein translation, and the unfolded protein response. The DIS3 (also called RRP44) gene harbored mutations in 4 out of 38 patients and the mutations were clustered within the RNB domain facing the enzyme's catalytic pocket. Based on the sequencing evidence, the authors genotyped an additional 161 multiple myeloma patients for the 12 most common BRAF mutations and found mutations in seven patients (4%). Patients with these mutations may benefit from treatment with BRAF inhibitors.

Illumina technology: Genome Analyzer$_{II}$

Identification of novel SNPs by next-generation sequencing of the genomic region containing the APC gene in colorectal cancer patients in China

Cheng Y, Wang J, Shao J, Chen Q, Mo F, et al. (2010) OMICS 14: 315–25.

Through SNP discovery in selected genomic regions of key genes in 27 pairs of colorectal cancers and normal adjacent tissues, 69 novel SNPs in the 123-kb APC genomic region were identified. Eleven SNPs are located in the exonic region, including one novel SNP. Ten of the SNPs are synonymous, but were predicted to affect splicing by creating or removing exonic splicing enhancers or exonic splicing silencers. The authors also identified seven SNPs in the upstream region of the APC gene, three of which were unique to the cancer tissues.

Illumina technology: Genome Analyzer

Genetic variation in glutathione metabolism and DNA repair genes predicts survival of small-cell lung cancer patients

Sun Z, Chen J, Aakre J, Marks R, Garces Y, et al. (2010) Ann Oncol 21: 2011–6.

In this study, 248 patients with primary small-cell lung cancer were genotyped for 419 tag SNPs in 49 genes in the glutathione and DNA repair pathways. After analysis at single-SNP, whole-gene, and haplotype levels, 21 SNPs in 11 genes were found to be significantly associated with patient survival. Whole-gene and haplotype analysis identified haplotype combinations and genomic locations underlying the observed SNP associations in 3 of the 11 genes.

Illumina technology: GoldenGate Genotyping Assay

Additional References

- Genetic variations of the PI3K-AKT-mTOR pathway and clinical outcome in muscle invasive and metastatic bladder cancer patients
  Chen M, Gu J, Delclos GL, Killary AM, Fan Z, et al. (2010) Carcinogenesis 31(8): 1387–1391.
- Distinct patterns of 1p and 19q alterations that have different prognoses identify subtypes of human gliomas
  Vogazianou AP, Chan R, Backlund LM, Pearson DM, Liu L, et al. (2010) Neuro Oncol.
- Prostate cancer genes associated with TMPRSS2-ERG gene fusion and prognostic of biochemical recurrence in multiple cohorts
  Barwick BG, Abramovitz M, Kodani M, Moreno CS, Nam R, et al. (2010) Br J Cancer 102(3): 570–576.
- Implementing prognostic and predictive biomarkers in CRC clinical trials
  Van Schaeybroeck S, Allen WL, Turkington RC and PG Johnston (2011) Nat Rev Clin Oncol 8(4): 222–232.
- Genetic variations in regulator of G-protein signaling genes as susceptibility loci for second primary tumor/recurrence in head and neck squamous cell carcinoma
  Wang J, Lippman S, Lee JJ, Yang H Khuri FR, et al. (2010) Carcinogenesis 31(10): 1755–1761.

Cancer Treatment

There is little doubt that genomic medicine will ultimately play a significant role in the diagnosis and treatment of cancer. For genetic medicine to be successful, we need a thorough understanding of the structure and function of the human genome and about the genetic components of human health and disease.

Evolution of an adenocarcinoma in response to selection by targeted kinase inhibitors

Jones SJ, Laskin J, Li YY, Griffith OL, An J, et al. (2010) Genome Biology 11: R82.

A fully integrated sequencing analysis of genomic DNA, mRNA-Seq, and CNV-Seq was used to inform patient treatment on a rare cancer where no standard protocols exist. The authors follow the primary tumor, before and after treatment, as well as in metastasis. A recurring metastasis possessed 7,288 genes within copy number amplicons, 385 genes exhibiting increased expression relative to other tumors, and 9 new somatic protein coding mutations. The observed mutations and amplifications were consistent with therapeutic resistance arising through activation of the MAPK and AKT pathways. The results provide direct in vivo genomic evidence for mutational evolution within a tumor under drug selection and potential mechanisms of drug resistance accrual.

Illumina technology: Genome Analyzer

Genetic diagnosis of familial breast cancer using clonal sequencing

Morgan JE, Carr IM, Sheridan E, Chu CE, Hayward B, et al. (2010) Hum Mutat 31: 484–491.

The authors compare the sensitivity and specificity of sequencing by synthesis (SBS) to current diagnostic methods to detect TP53, BRCA1, and BRCA2 mutations in established tumor cell lines and patient samples. All known pathogenic mutations (including point mutations and insertions/deletions of up to 16 nucleotides) were detected, leading to the conclusion that SBS outperforms current diagnostic methods. In addition, SBS provided reduced analysis times and reagent costs compared to Sanger sequencing. These improvements open the possibility of BRCA1/2 testing for a wider spectrum of at-risk women, and will allow the genetic classification of tumors prior to the use of novel PARP inhibitors to treat BRCA-deficient breast cancers.

Illumina technology: Genome Analyzer

Detection of inherited mutations for breast and ovarian cancer using genomic capture and massively parallel sequencing

Walsh T, Lee MK, Casadei S, Thornton AM, Stray SM, et al. (2010) Proc Natl Acad Sci U S A 107: 12629–33.

The authors developed a sequencing-based genomic assay to detect all mutations in 21 genes, including BRCA1 and BRCA2, in which inherited mutations are known to predispose women to breast or ovarian cancer. The average coverage was > 1,200 reads per base pair. In 20 cases tested there were zero false-positive calls of nonsense mutations, frameshift mutations, or genomic rearrangements for any gene in any of the test samples. In addition, the test is able to detect de novo mutations in these genes.

Illumina technology: Genome Analyzer$_{IIx}$

IDH2 somatic mutations in chronic myeloid leukemia patients in blast crisis

Soverini S, Score J, Iacobucci I, Poerio A, Lonetti A, et al. (2010) Leukemia 25: 178–81.

Cancer progression, treatment, and outcome were tracked in a chronic myeloid leukemia (CML) patient. An IDH2 R140Q heterozygous mutation deriving from a G to A nucleotide substitution on chromosome 15, position 88432938 (hg18, NCBI build 36.1) was found in the sample collected at the time of progression to lymphoid blast crisis, but this variant was not seen in the sample collected at diagnosis nor in remission. This study offers a glimpse of how markers could be developed to track disease progression.

Illumina technology: Genome Analyzer$_{II}$

Reviews

Charting a course for genomic medicine from base pairs to bedside

Green ED, Guyer MS, Manolio TA and JL Peterson (2011) Nature 470: 204–13.

This review represents a vision for the future of genomics. It describes the path towards an era of genomic medicine, and discusses the imperatives of genomics-based diagnostics routine, defining the genetic components of disease, comprehensive characterization of cancer genomes, developing practical systems for clinical genomic informatics, and understanding the role of the human microbiome in health and disease.

The molecular pathology of cancer

Harris TJ and F McCormick (2010) Nat Rev Clin Oncol 7: 251–65.

A comprehensive review of how DNA sequencing and genome-wide association studies are driving discovery of germline and somatic mutations present in different cancers. The authors focus on how disease subtypes can influence therapy and discuss the implications of the impending molecular diagnostic revolution from the point of view of patients, clinicians, and diagnostic and pharmaceutical companies.

Additional References

- Clinical implications of next-generation sequencing for cancer medicine
  Shuen A and WD Foulkes (2010) Curr Oncol 17: 39–42.
- Sequencing firms vie for diagnostics market, tiptoe round patents
  Eisenstein M (2010) Nat Biotechnol 28(7): 635–636.
- Making breast cancer risk assessment personal
  Vanchieri C (2010) J Natl Cancer Inst 102(13): 924–926.
- Pharmacy benefit managers, pharmacies, and pharmacogenomic testing: Prescription for progress?
  Topol EJ (2010) Sci Transl Med 2(44): 44.

General Reviews of Cancer Genomics

- Advances in understanding cancer genomes through second-generation sequencing
  Meyerson M, Gabriel S and G Getz (2010) Nat Rev Genet 11: 685–96.
- High-throughput DNA sequencing—concepts and limitations
  Kircher M and J Kelso (2010) Bioessays 32: 524–36.
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  K Robison (2010) Brief Bioinform 11: 524–534.

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