Genomic Profiling of Liver Cancer

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Development of liver cancers is driven largely by genomic alterations that deregulate signaling pathways, influencing growth and survival of cancer cells. Because of the hundreds or thousands of genomic/epigenomic alterations that have accumulated in the cancer genome, it is very challenging to find and test candidate genes driving tumor development and progression. Systematic studies of the liver cancer genome have become available in recent years. These studies have uncovered new potential driver genes, including those not previously known to be involved in the development of liver cancer. Novel approaches combining multiple datasets from patient tissues have created an unparalleled opportunity to uncover potential new therapeutic targets and prognostic/predictive biomarkers for personalized therapy that can improve clinical outcomes of the patients with liver cancer.

Keywords: gene expression profiling, liver neoplasms, oligonucleotide array sequence analysis

Introduction

Primary liver cancer is one of the most common cancers in the world, accounting for an estimated 600,000 deaths annually [1]. In Korea, liver cancer is the second leading cause of cancer-related deaths (10,946 death in 2010) [2]. In the United States, primary liver cancer has gained major interest, because the incidence of liver cancer has increased over the past 25 years, and the incidence and mortality rate of liver cancer are expected to double over the next 10 to 20 years [3-5]. Therefore, prevention and treatment of liver cancer are of great concern.

While primary liver cancer is one of the few cancers with well-defined major risk factors, such as hepatitis virus infection, alcohol consumption, and obesity, the molecular pathogenesis of liver cancer is not well understood [6-8]. Patients with liver cancer have a highly variable clinical course [7, 9], indicating that liver cancer comprises several biologically distinct subgroups. Therefore, it will be necessary to understand liver cancer at a genomic and molecular level for improved stratification of patients, identification of novel druggable targets, and development of personalized treatment, based on the biological characteristics of tumors in each patient.

Due to the complex nature of cancers that are initiated by activation of many different oncogenes or inactivation of different tumor suppressor genes and progress by additional genetic or epigenetic alterations, conventional gene-by-gene approaches are likely to deliver only a limited understanding of the biological and pathological characteristics of cancer cells. However, the recent development of technologies for gene expression profiling, genome-wide copy number analysis, and whole-genome sequencing has enabled the comprehensive characterization of entire cancer genomes. This information promises to improve our understanding of the genetic and epigenetic alterations driving the development of liver cancer, as well as ultimately provide guidance on personalized treatment of patients. Although these large-scale genomic data promise improvement in the treatment of liver cancer, challenges remain for the management and integration of these data for a better understanding of how these alterations give rise to the development of liver cancer and, most importantly, the translation of these findings into personalized patient treatment.

Approaches for Molecular Profiling of Liver Cancer

Comparative genomic hybridization (CGH)

Since the discovery of frequent genetic rearrangements...
and copy number aberrations in the cancer genome [10],
cytogenetic methods have been used widely to find the
genetic changes in cancer genomes. The CGH method was
the first tool to provide a genome-wide investigation of copy-
number alterations in cancer [11]. Increased or decreased
regions of copy number are believed to have either onco-
genes or tumor suppressor genes. Although the resolution
of early CGH mapping technology is limited by 2 Mbp (high-
copy-number amplifications) to 10 Mbp (low-copy-number
amplification or deletion), this method has identified many
loci for oncogenes and tumor suppressor genes in liver
cancer. Multiple studies have reported amplification of the
8q24 loci, in which MYC is located [12-15]. Other studies
also identified prevalent amplification of the 1q, 6p, and 17q
regions and frequent deletion of the 8p, 16q, 4q, and 17p
regions [12, 15].

Microarray-based technologies

Microarray technology has been used extensively to
gather global-scale data from cell lines or tissues of interest.
This technology was first used to make a quantitative assess-
ment of mRNAs or non-coding RNAs. Gene expression
profiling studies based on microarray technologies have
identified conserved gene expression signatures that are
significantly related with clinical outcomes or pathological
phenotypes and have uncovered many subgroups of cancer
that were not recognized with conventional approaches in a
variety of cancers [16-18]. These approaches have been used
successfully to identify therapeutic targets for various
cancers and to predict overall survival or recurrence-free
survival rates of cancer patients [19-21].

Use of microarray technology is not restricted to genome-
wide gene expression profiling of cells or tissues but expands
to uncovering single-nucleotide polymorphisms associated
with cancer risk, methylation status of gene promoters,
DNA copy number alterations, protein expression profiling,
and re-sequencing of cancer genomes. Because microarray-
based CGH can provide copy-number analyses with much
higher resolution than conventional CGH, it has quickly
replaced conventional CGH [22, 23].

Microarray technologies were also adopted by the prote-
omics community. Protein microarray was quickly estab-
lished by rapid improvements to miniaturizing western
blotting and tissue lysate dotting technologies onto solid
surfaces. There are two different experimental approaches
for protein arrays: forward-phase protein array (FPPA) and
reverse-phase protein array (RPPA). In FPPA, antibodies or
polypeptides are printed on the slide surface, and each
slide is incubated with labeled tissue or cell lysate. This
approach allows one to simultaneously measure multiple
protein features, such as expression, interaction with
peptide ligands, and protein phosphorylation, from the
samples. In RPPA, an individual tissue lysate in printed on
the slide surface, and each RPPA slide consists of hundreds
or thousands of tissue samples. Each slide is then probed
with one peptide ligand or antibody at a time, and a protein
feature of interest is assessed and compared across many
tissues or cell lines. Because RPPA needs only several
thousands of cells to collect high-quality data, it is better
suited for cancer research. In contrast, FPPA is not suitable
for tissue-based studies, because it needs a large quantity of
tissue or cell lysate for detection [24-27].

Next-generation sequencing

Due to the emergence of second-generation sequencing
technologies that allow massive parallel collection of se-
quence information, the cost per base for sequencing the
entire genome has reduced dramatically [28]. These new
technologies provided unique opportunities to investigate
all sequences of entire cancer genomes to uncover the
genetic changes that arise during cancer development. Many
different technologies have been developed by different
companies: Roche Applied Science (454 Genome Sequencer
FLX System; Indianapolis, IN, USA), Life Technologies
(Sequencing by Oligonucleotide Ligation and Detection or
SOLiD; Carlsbad, CA, USA), Illumina (Genome Analyzer II;
San Diego, CA, USA), Helicos BioSciences (HeliScope Single
Molecule Sequencer; Cambridge, MA, USA), and Ion Torrent
Systems (now owned by Life Technologies, Ion Proton
System).

These new technologies have been used to identify poten-
tial driver mutations in the development of liver cancer
through whole-genome and -exome sequencing. The first
sequencing of the primary liver cancer genome revealed a
total of 11,731 somatic mutations [29]. Following studies
showed that the CTNNB1, TP53, and EGFR genes were
frequently mutated in liver cancer [30-33]. Additionally, the
ARID family, including ARID1A, ARID1B, and ARID2, was
mutated in about half of all tumors, suggesting a potential
role of them in the development of liver cancer.

Gene Expression Profiling of Liver Cancer

In previous studies [18, 34-37], gene expression data from
human liver cancer have identified several subgroups that
are significantly associated with clinical outcomes, such as
overall survival and recurrence-free survival. These findings
strongly indicated that gene expression patterns faithfully
reflect biological and pathological heterogeneity among
patients with liver cancer and would be highly valuable in
predicting the clinical outcome of patients. The current
challenge for clinical use is to identify those who will not
benefit from conventional treatments and to offer alternative therapies. If master drivers, such as genes or pathways determining the biological characteristics of the tumors, can be identified, we can explore their therapeutic potentials. However, gene expression profiling approaches are also limited, because they only provide information related to transcriptional regulation.

**Integromics: Integration of multiple-omics data**

Previous studies undoubtedly showed that gene expression patterns (or signatures) are useful markers that can stratify patients and offer useful prognostic information [18, 37-42]. The current research focus has moved to uncovering genetic/epigenetic factors that are key regulators of specific signaling pathways altered in malignant cancer cells, potentially guiding the discovery of new therapeutic (and druggable) targets [8, 43-45]. But, the selection of such candidates for future validations from long gene lists produced by genome-wide screening is a significant challenge because of potential confounding factors embedded in many genome-wide data from human cancers. Furthermore, because the gene expression data from tumor tissues only provide “snapshot” information of genetic/epigenetic alterations and lack in-depth information on interactive and time-dependent alterations during tumor progression, it is not easy to distinguish the driver genes from passenger genes whose alterations simply echo changes in cell proliferation or organ physiology.

Integrative approaches for systematic analysis of copy number alteration data and gene expression from the same patients have been proposed to identify potential driver genes [46]. This study demonstrated that DNA copy number alteration data provide additional prognostic significance. Integrative analysis of copy number alteration data and gene expression further identified 50 driver candidates whose activation was triggered by copy number amplifications of genes and also significantly associated with aggressive tumor phenotypes in liver cancer.

Alterations in DNA copy number and expression patterns of thousands of genes are important characteristics of many cancer cells. Because the use of high-throughput microarray-based technologies, mass spectrometry, and second-generation sequencers for the analysis of cancer genomes inevitably produces many false-positive results, it is not easy to select practical numbers of candidate genes for future evaluation of them as therapeutic targets and/or prognostic/predictive biomarkers. Therefore, it is important to combine two or more genomic scale datasets (e.g., array-based CGH data, promoter methylation, and expression of coding or non-coding genes), collected independently from the same tissues (Fig. 1).

RPPA is a newly established proteomic technology [24-27] that provides quantitative assessment of the expression and modification of many proteins. For example, the phosphorylation level of particular proteins can be estimated easily by using antibodies specific to certain phosphorylation sites of proteins. Use of the phosphorylation antibodies allows us to assess the signaling pathways by looking at many kinase substrates simultaneously through multiplexed phosphorylation-specific antibody analysis. However, assessment of signaling pathways by RPPA is restricted by the availability of antibodies with very high specificity. This limitation can be overcome by integration of multiple datasets for systematic analysis. Integration of proteomic data with genomic data would significantly help us have better insights into tumor development and progression. Furthermore, contributor genes (or drivers) will be quickly identified by integration of proteomic data with one or more genomic datasets.

**The Cancer Genome Atlas (TCGA) Project: new opportunity for cancer genomics**

TCGA is a landmark research program supported by the National Human Genome Research Institute and National Cancer Institute at the National Institutes of Health in the
Table 1. Cancer types investigated by the TCGA project

| Available cancer types                        | Cases with data | Date last updated |
|-----------------------------------------------|-----------------|-------------------|
| Acute myeloid leukemia                        | 200             | Sep 10, 2013      |
| Adrenocortical carcinoma                      | 92              | Oct 14, 2013      |
| Bladder urothelial carcinoma                  | 212             | Oct 18, 2013      |
| Brain lower grade glioma                      | 307             | Oct 18, 2013      |
| Breast invasive carcinoma                     | 1,009           | Oct 18, 2013      |
| Cervical squamous cell carcinoma              | 165             | Oct 18, 2013      |
| Colon adenocarcinoma                          | 438             | Oct 16, 2013      |
| Esophageal carcinoma                          | 64              | Oct 18, 2013      |
| Glioblastoma multiforme                       | 510             | Oct 11, 2013      |
| Head and Neck squamous cell carcinoma         | 458             | Oct 16, 2013      |
| Kidney chromophobe                            | 66              | Oct 18, 2013      |
| Kidney renal clear cell carcinoma             | 512             | Oct 18, 2013      |
| Kidney renal papillary cell carcinoma         | 158             | Oct 18, 2013      |
| Liver hepatocellular carcinoma                | 152             | Oct 18, 2013      |
| Lung adenocarcinoma                           | 499             | Oct 14, 2013      |
| Lung squamous cell carcinoma                  | 493             | Oct 18, 2013      |
| Diffuse large B-cell lymphoma                 | 23              | Oct 18, 2013      |
| Ovarian serous cystadenocarcinoma             | 570             | Oct 18, 2013      |
| Pancreatic adenocarcinoma                     | 67              | Oct 16, 2013      |
| Prostate adenocarcinoma                       | 251             | Oct 15, 2013      |
| Rectum adenocarcinoma                         | 168             | Oct 18, 2013      |
| Sarcoma                                       | 114             | Oct 18, 2013      |
| Skin cutaneous melanoma                       | 360             | Oct 15, 2013      |
| Stomach adenocarcinoma                        | 340             | Oct 14, 2013      |
| Thyroid carcinoma                             | 500             | Oct 17, 2013      |
| Uterine carcinosarcoma                        | 57              | Oct 14, 2013      |
| Uterine corpus endometrial carcinoma          | 514             | Oct 18, 2013      |

TCGA, The Cancer Genome Atlas.

United States that was started as a pilot project in 2006 and expanded later in 2009. The goal of TCGA is to collect comprehensive genomic and proteomic information on all major human cancers, including liver cancer. Initial efforts focused on glioblastoma, lung squamous cell cancer, and ovarian cancer as pilot projects [47-50]. By using various different platforms, TCGA currently gathers many different genome-wide data, including mRNA expression, microRNA expression, somatic mutations, copy number alterations, and promoter methylation. In addition, it also generates proteomic data by using RPPA technology. The project plans to collect genomic and proteomic data from more than 500 tissues per cancer type and release the data to the public without any restriction in use of the data (Table 1).

This ambitious project has identified novel driver genes and biomarkers on the basis of genomic, transcriptomic, proteomic, and epigenomic alterations. Some findings are clinically relevant and unexpected. For example, we have now learned that non-hypermutated adenocarcinomas of the colon and rectum are not distinguishable at the genomic level [51]. In lung squamous cell cancer, while KRAS and EGFR mutations, the most commonly activated oncogenes in lung adenocarcinoma, are extremely rare, alterations in the FGFR kinase family are common [49]. Thus, massive data from a large number of tissues have created an unprecedented opportunity for taking an integrated approach toward a systems-level understanding of disruptions in cellular and molecular pathways in cancer.

Conclusion

Genomics has become a dominant tool for understanding cancer biology, revealing unexpected surprises and providing cancer classification according to biological differences. The amount of liver cancer-related genomic data that have generated during the past few years is really incredible. Analysis approaches of these data have been advanced to efficiently process this information and allow us to perform integrated genome-wide analysis on a scale previously unthinkable. These approaches are also beginning to investigate the dense network of molecular pathways driving the development of liver cancer. The anticipated benefits of these data will be more a faithful assessment of prognosis than the current stage system, improved prediction of treatment response, and discovery of new therapeutic targets. Future challenges lie in translating this discovery into personalized treatment for the patient with liver cancer.

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References

1. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin 2005;55:74-108.
2. Jung KW, Won YJ, Kong HJ, Oh CM, Seo HG, Lee JS. Cancer statistics in Korea: incidence, mortality, survival and prevalence in 2010. Cancer Res Treat 2013;45:1-14.
3. Davila JA, Morgan RO, Shaib Y, McGlynn KA, El-Serag HB. Hepatitis C infection and the increasing incidence of hepatocellular carcinoma: a population-based study. Gastroenterology 2004;127:1372-1380.
4. El-Serag HB, Mason AC. Rising incidence of hepatocellular carcinoma in the United States. N Engl J Med 1999;340:745-750.
5. El-Serag HB. Hepatocellular carcinoma: recent trends in the United States. Gastroenterology 2004;127(S Suppl 1):S27-S34.
6. Bruix J, Boix L, Sala M, Llovet JM. Focus on hepatocellular
carcinoma. Cancer Cell 2004;5:215-219.

7. Llovet JM, Burroughs A, Bruix J. Hepatocellular carcinoma. \textit{Lancet} 2003;362:1907-1917.

8. Thorgeirsson SS, Grisham JW. Molecular pathogenesis of human hepatocellular carcinoma. \textit{Nat Genet} 2002;31:339-346.

9. Llovet JM, Bustamante J, Castells A, Vilana R, Ayuso Mdel C, Sala M, \textit{et al.} Natural history of untreated nonsurgical hepatocellular carcinoma: rationale for the design and evaluation of therapeutic trials. \textit{Hepatology} 1999;29:62-67.

10. Nowell PC, Hungerford DA. Chromosome studies on normal and leukemic human leukocytes. \textit{J Natl Cancer Inst} 1960;25:85-109.

11. Kallioniemi A, Kallioniemi OP, Sudar D, Rutovitz D, Gray JW, Waldman F, \textit{et al.} Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. \textit{Science} 1992;258:818-821.

12. Moinzadeh P, Breuhahn K, Stützer H, Schirmacher P. Chromosome alterations in human hepatocellular carcinoma correlate with aetiology and histological grade: results of an explorative CGH meta-analysis. \textit{Br J Cancer} 2005;92:935-941.

13. Kusano N, Shiraishi K, Kubo K, Oga A, Okita K, Sasaki K. Genetic aberrations detected by comparative genomic hybridization in hepatocellular carcinoma: their relationship to clinicopathological features. \textit{Hepatology} 1999;29:1858-1862.

14. Zimonjic DB, Keck CL, Thorgeirsson SS, Popescu NC. Novel recurrent genetic imbalances in human hepatocellular carcinoma cell lines identified by comparative genomic hybridization. \textit{Hepatology} 1999;29:1208-1214.

15. Poon TC, Wong N, Lai PB, Rattray M, Johnson PJ, Sung JJ. A tumor progression model for hepatocellular carcinoma: bioinformatic analysis of genomic data. \textit{Gastroenterology} 2006;131:1262-1270.

16. Alizadeh AA, Eisen MB, Davis RE, Ma C, Lossos IS, Rosenwald A, \textit{et al.} Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. \textit{Nature} 2000;403:503-511.

17. Bittner M, Meltzer P, Chen Y, Jiang Y, Seftor E, Hendrix M, \textit{et al.} Molecular classification of cutaneous malignant melanoma by gene expression profiling. \textit{Nature} 2000;406:536-540.

18. Lee JS, Chu IS, Heo J, Calvisi DF, Sun Z, Roskams T, \textit{et al.} Classification and prediction of survival in hepatocellular carcinoma by gene expression profiling. \textit{Hepatology} 2004;40:667-676.

19. Beer DG, Kardia SL, Huang CC, Giordano TJ, Levin AM, Misek DE, \textit{et al.} Gene-expression profiles predict survival of patients with lung adenocarcinoma. \textit{Nat Med} 2002;8:816-824.

20. van’t Veer LJ, Dai H, van de Vijver MJ, He YD, Hart AA, Mao M, \textit{et al.} Gene expression profiling predicts clinical outcome of breast cancer. \textit{Nature} 2002;415:530-536.

21. van de Vijver MJ, He YD, van’t Veer LJ, Dai H, Hart AA, Voskuil DW, \textit{et al.} A gene-expression signature as a predictor of survival in breast cancer. \textit{N Engl J Med} 2002;347:1999-2009.

22. Pinkel D, Segraves R, Sudar D, Clark S, Poole I, Kowbel D, \textit{et al.} High resolution analysis of DNA copy number variation using comparative genomic hybridization to microarrays. \textit{Nat Genet} 1998;20:207-211.

23. Pollack JR, Perou CM, Alizadeh AA, Eisen MB, Pergamenschikov A, Williams CF, \textit{et al.} Genome-wide analysis of DNA copy-number changes using cDNA microarrays. \textit{Nat Genet} 1999;23:41-46.

24. Sheehan KM, Calvert VS, Kay EW, Lu Y, Fishman D, Espina V, \textit{et al.} Use of reverse phase protein microarrays and reference standard development for molecular network analysis of metastatic ovarian carcinoma. \textit{Mol Cell Proteomics} 2005;4:346-355.

25. Espina V, Mehta AI, Winters ME, Calvert V, Wulffahle J, Petricoin EF 3rd, \textit{et al.} Protein microarrays: molecular profiling technologies for clinical specimens. \textit{Proteomics} 2003;3:2091-2100.

26. Haab BB. Antibody arrays in cancer research. \textit{Mol Cell Proteomics} 2005;4:377-383.

27. Liotta LA, Espina V, Mehta AI, Calvert V, Rosenblatt K, Geho D, \textit{et al.} Protein microarrays: meeting analytical challenges for clinical applications. \textit{Cancer Cell} 2003;3:317-325.

28. Davie JW, Hohenlohe PA, Etter PD, Boone JQ, Catchen JM, Blaxter ML. Genome-wide genetic marker discovery and genotyping using next-generation sequencing. \textit{Nat Rev Genet} 2011;12:499-510.

29. Totoki Y, Tatsuno K, Yamamoto S, Arai Y, Hosoda F, Ishikawa S, \textit{et al.} High-resolution characterization of a hepatocellular carcinoma genome. \textit{Nat Genet} 2011;43:464-469.

30. Li M, Zhao H, Zhang X, Wood LD, Anders RA, Choti MA, \textit{et al.} Inactivating mutations of the chromatin remodeling gene ARID2 in hepatocellular carcinoma. \textit{Nat Genet} 2011;43:828-829.

31. Fujimoto A, Totoki Y, Abe T, Boroevich KA, Hosoda F, Nguyen HH, \textit{et al.} Whole-genome sequencing of liver cancers identifies etiological influences on mutation patterns and recurrent mutations in chromatin regulators. \textit{Nat Genet} 2012;44:760-764.

32. Guichard C, Amaddeo G, Imbeaud S, Ladeiro Y, Pelletier L, Maad IB, \textit{et al.} Integrated analysis of somatic mutations and focal copy-number changes identifies key genes and pathways in hepatocellular carcinoma. \textit{Nat Genet} 2012;44:694-698.

33. Sung WK, Zheng H, Li S, Chen R, Liu X, Li Y, \textit{et al.} Genome-wide survey of recurrent HBV integration in hepatocellular carcinoma. \textit{Nat Genet} 2012;44:765-769.

34. Hoshida Y, Villanueva A, Kobayashi M, Peix J, Chiang DY, Camargo A, \textit{et al.} Gene expression in fixed tissues and outcome in hepatocellular carcinoma. \textit{N Engl J Med} 2008;359:1955-1965.

35. Ninte JS, Heo J, Libbrett L, Chu IS, Kaposi-Novak P, Calvisi DF, \textit{et al.} A novel prognostic subtype of human hepatocellular carcinoma derived from hepatic progenitor cells. \textit{Nat Med} 2006;12:410-416.
38. Lee JS, Thorgeirsson SS. Functional and genomic implications of global gene expression profiles in cell lines from human hepatocellular cancer. *Hepatology* 2002;35:1134-1143.

39. Lee JS, Chu IS, Mikaelyan A, Calvisi DF, Heo J, Reddy JK, et al. Application of comparative functional genomics to identify best-fit mouse models to study human cancer. *Nat Genet* 2004;36:1306-1311.

40. Lee JS, Thorgeirsson SS. Genome-scale profiling of gene expression in hepatocellular carcinoma: classification, survival prediction, and identification of therapeutic targets. *Gastroenterology* 2004;127(5 Suppl 1):S51-S55.

41. Lee JS, Grisham JW, Thorgeirsson SS. Comparative functional genomics for identifying models of human cancer. *Carcinogenesis* 2005;26:1013-1020.

42. Lee JS, Thorgeirsson SS. Genetic profiling of human hepatocellular carcinoma. *Semin Liver Dis* 2005;25:125-132.

43. Thorgeirsson SS, Lee JS, Grisham JW. Functional genomics of hepatocellular carcinoma. *Hepatology* 2006;43(2 Suppl 1):S145-S150.

44. Thorgeirsson SS, Lee JS, Grisham JW. Molecular prognostication of liver cancer: end of the beginning. *J Hepatol* 2006;44:798-805.

45. Thorgeirsson SS. Genomic decoding of hepatocellular carcinoma. *Gastroenterology* 2006;131:1344-1346.

46. Woo HG, Park ES, Lee JS, Lee YH, Ishikawa T, Kim YJ, et al. Identification of potential driver genes in human liver carcinoma by genomewide screening. *Cancer Res* 2009;69:4059-4066.

47. Cancer Genome Atlas Research Network. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature* 2008;455:1061-1068.

48. Cancer Genome Atlas Research Network. Integrated genomic analyses of ovarian carcinoma. *Nature* 2011;474:609-615.

49. Cancer Genome Atlas Research Network. Comprehensive genomic characterization of squamous cell lung cancers. *Nature* 2012;489:519-525.

50. International Cancer Genome Consortium, Hudson TJ, Anderson W, Artez A, Barker AD, Bell C, et al. International network of cancer genome projects. *Nature* 2010;464:993-998.

51. Cancer Genome Atlas Network. Comprehensive molecular characterization of human colon and rectal cancer. *Nature* 2012;487:330-337.