Implementation of individualized medicine for cancer patients by multiomics-based analyses—the Project HOPE—

Ken Yamaguchi1, 2, Kenichi Ura Kami3, Keiichi Ohshima4, Tohu Mochizuki4, Yasuto Akiyama5, Katsuhiko Uesaka1, Takashi Nakajima1, Mitsuru Takahashi1, Sunao Tama1, and Masatoshi Kusuhara6, 7
1 Shizuoka Cancer Center Hospital, Shizuoka, Japan; 2 Shizuoka Cancer Center Research Institute, Shizuoka, Japan; 3 Cancer Diagnostics Research Division, Shizuoka Cancer Center Research Institute, Shizuoka, Japan; 4 Medical Genetics Division, Shizuoka Cancer Center Research Institute, Shizuoka, Japan; 5 Immunotherapy Division, Shizuoka Cancer Center Research Institute, Shizuoka, Japan; 6 Regional Resources Division, Shizuoka Cancer Center Research Institute, Shizuoka, Japan; and 7 Drug Discovery & Development Division, Shizuoka Cancer Center Research Institute, Shizuoka, Japan

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ABSTRACT

The Project HOPE (High-tech Omics-based Patient Evaluation) for cancer medicine aims to evaluate biological characteristics of each cancer tissue as well as diathesis of each patient in around 1,000 consecutive cases per year, who receive operations at the Shizuoka Cancer Center. Cancer tissues are investigated by whole-exome sequencing for 18,835 genes, focusing on 12,776 in-house cancer hotspots from 483 cancer-associated genes. To confirm cancer-specific genetic changes, we analyzed blood cells to collate with data of cancer tissues, and we reevaluate cancer tissues by comprehensive cancer panel for 409 genes. In order to investigate diathesis of the patients, we evaluate 43,015 hotspots associated with non-cancerous diseases. In terms of gene expression profiling, we analyze cancer-specific alterations for 29,833 genes using tumor and adjacent normal tissues. If and when necessary, we investigate tumor and normal tissues by proteomics and metabolomics. The model experiments using glioblastoma cell lines demonstrated that the method is appropriate for clinical application. The Project HOPE makes it possible to implement individualized medicine and to practice preventive and presymptomatic medicine for cancer patients. Furthermore, the project can create important seeds for research and development in cancer medicine.

1. Preface and the project design

Recent progress in cancer research and molecular biology has revealed that abnormalities in signal transduction pathway in cell growth play a critical role for the development of cancer cells (5, 7, 20, 21). Strong evidences have accumulated to prove that cancer cells possess their own driver genes and produce factors with activities to modulate surrounding microenvironment, which explains cancer cell growth and metastases. Since these genetic changes could be molecular targets for anti-cancer drugs, clinicians as well as researchers explore appropriate use of genetic data in clinical practice for cancer patients.

The Shizuoka Cancer Center started the Project HOPE (High-tech Omics-based Patient Evaluation) for cancer medicine in late January, 2014. This project aims to evaluate biological characteristics of cancer and diathesis of each patient by multiomics-based analyses, which integrate genomics, transcriptomics, proteomics and metabolomics. The research plan was designed according to the revised Ethical Guidelines for Human Genome/Gene Analysis Research in Japan (17) and was approved by the Institutional Review Board of the Shizuoka Cancer Center.
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Center. Only patients giving written informed consent participate in the study. For protecting anonymities of the patients, collaboration among surgeons, anesthesiologists, pathologists and the staff at the Patient’s Information Protection Office plays an important role.

The patients being studied are about one third of those who take surgeries to remove cancers at the Shizuoka Cancer Center Hospital, and are able to supply fresh and enough quantity of cancer tissues of approximately 0.1 g or greater each. In this criterion, the number of patients enrolled is assumed to reach around 1,000 per year, and around 3,000 during the 3-year research period. During the initial 6-month period, about 500 cancer patients were enrolled, which indicates that the study is on a good pace.

Fig. 1 describes the experimental design of the Project HOPE. We obtained blood cells, tumor tissue and adjacent normal tissue from each patient at the time of operation. Blood cells and tumor tissue are analyzed by whole-exome sequencing. DNA is extracted by the method reported previously (1). Using 100 ng of DNA, whole exons are amplified with AmpliSeq Exome kit (Life Technologies, CA, USA) (11), and sequencing is performed by next-generation sequencer of Ion Proton platform (Life Technologies); in total, 293,903 DNA pieces of whole exons from 18,835 genes are amplified and then applied to the next-generation sequencer. In average, around 100 amplicons of each DNA piece are evaluated and then sequenced; when we obtain only 20 amplicons or less, we consider the data as unreliable and discard it. By this criterion, 95% of exon-derived DNA pieces were analyzed successfully. To confirm the results of whole-exome sequencing, tumor tissues are reevaluated by the Comprehensive Cancer Panel for 409 genes (Life Technologies) (15).

Fig. 1. The experimental design of the Project HOPE. From each patient, we obtain blood cells, tumor tissue and adjacent normal tissue, which are evaluated by multiomics-based analyses. Blood cells and tumor tissue are analyzed by whole-exome sequencing. From the results of blood cell analyses, risk for life-style diseases and inheritable diseases could be evaluated, which in turn make it possible to achieve “preventive and presymptomatic medicine” or “Mibyo medicine”. Cancer-specific genetic changes including single nucleotide variances and inserts/deletions are decided by collation with reference standard and data from blood cells. Tumor tissues along with the surrounding normal tissues are examined by gene expression profiling. Cancer-specific alterations in gene expression are evaluated by collation of the expression level of tumor tissue with that of adjacent normal tissue. If and when necessary, we investigate tumor and normal tissues by proteomics and metabolomics, as described by the dotted lines. Cancer-specific genetic changes, cancer-specific alterations in gene expression and cancer-specific alterations in proteins and metabolites are useful for implementing individualized medicine for cancer patients.
Data analyses of whole-exome sequencing have been focused on 12,776 in-house cancer hotspots (HOPE Cancer Hotspots) from 483 cancer-associated genes, which were collected from 3 data sets: the Cancer Comprehensive Panel (Life Technologies) (15), the FoundationOne (Foundation Medicine, MA, USA) (4), and lists of driver genes proposed by Vogelstein et al. (20). All of these hotspots are defined as “Confirmed somatic” and “Tumor sample” in the catalogue of somatic mutations in cancer version 68 (COSMIC v68) database (3). Moreover, we also plan to analyze 5,679 hotspots in 54 genes associated with hereditary cancer syndromes and 40,447 hotspots in 8,170 genes associated with non-cancerous diseases, which are obtained from ClinVar database published by the National Center for Biotechnology Information, USA (9).

Cancer-specific single nucleotide variances (SNVs) and inserts/deletions are decided by collation with reference standard and data from blood cells. Overexpression of genes and gene amplifications are analyzed by whole-exome sequencing and gene expression profiling as described later. Fusion genes generated by chromosomal translocation will be separately evaluated by the method using multiplex PCR combined with targeted fusion cDNA sequencing; in short, in-house fusion transcript primers are designed to amplify cDNA targets including breakpoints, and are amplified by multiplex PCR. The amplicons are then sequenced and analyzed.

From the results of analyses on data from blood cells, risk for life-style diseases and inheritable diseases could be evaluated, which in turn makes it possible to achieve preventive and presymptomatic medicine. This is also called “Mibyo medicine” in Japanese as described later.

We also evaluate cancer-specific alterations in gene expression. Tumor tissue and adjacent normal tissue are dissected from surgical specimens, and then stored in RNAlater (Life Technologies). Total RNA extraction and quality check are performed by the method reported previously (13), and the value of RNA integrity number equal to or greater than 6.0 is used for DNA microarray analysis. The total RNA extracted with a successful rate for recovery was 97.0% in 435 samples. One hundred nanograms of the total RNA is amplified and fluorescent-labeled using the Low Input Quick Amp Labeling Kit One Color (Agilent Technologies, CA, USA), according to the manufacturer’s instruction. The labeled sample is hybridized to the SurePrint G3 Human Gene Expression 8×60K v2 Microarray with 50,599 probes (Agilent Technologies), which can detect 29,833 genes registered in Entrez Gene Database published by the National Center for Biotechnology Information (16). The expression levels are calculated by the method described previously (8), and the data of tumor tissue is collated with that of adjacent normal tissue. When the gene expression level in the cancer tissue is increased 10 times or greater than that of normal tissue, the concerned gene is judged to be overexpressed. With regard to overexpressed genes, when whole-exome sequencing indicates multiplicity of DNA pieces of cancer-derived DNA being 10 times or greater than that of blood cells, the concerned gene is suspected to have a gene amplification.

If and when necessary, we analyze tumor and normal tissues by proteomics (8, 12, 13) and metabolomics (18, 19). Multimics-based analyses demonstrating cancer-specific genetic changes, cancer-specific alterations in gene expression and cancer-specific alterations in proteins and metabolites are useful for implementing individualized medicine for cancer patients.

2. Model experiments using cancer cell lines

As model experiments, we examined two commercially available glioblastoma cell lines, A172 and U-118-MG (14), by the method described above. In the case of cultured cell lines, normal control tissues were not available, and therefore, in the whole-exome sequencing, we compared the data with the reference standard alone. Thus, SNVs of the HOPE Cancer Hotspots were detected; 10 of these were also reported in the CCLP and the other two SNVs were detected only in the CCLP. In the case of cultured cell lines, normal control tissues were not available, and therefore, in the whole-exome sequencing, we compared the data with the reference standard alone. Thus, SNVs of the HOPE Cancer Hotspots in 483 cancer-associated genes were decided. The inserts/deletions, gene amplifications and fusion genes were not evaluated in the cultured cell line study.

Fig. 2 describes the results of whole-exome sequencing and gene expression profiling in two cell lines. Since both cell lines were examined by whole-exome sequencing in the COSMIC Cell Lines Project (CCLP) (2), the results of two projects were compared. In the case of A172 cell line, 15 SNVs in the HOPE Cancer Hotspots were detected; 10 of these were also reported in the CCLP and the other two SNVs were detected only in the CCLP. In the case of U-118-MG cell line, 15 SNVs were detected in the HOPE Cancer Hotspots; 9 of these were also reported in the CCLP, and other one SNV was detected only in the CCLP. It is reasonable to speculate that the cause of the difference between two studies could be resultant from different sensitivities in both methodologies as well as newly-developed SNVs during cell culture.
Recently, it was established that at least 13 signal transduction pathways were responsible for cancer cell proliferation (21), and in most of pathways, new drugs targeted for the pathways were developed (7). Fig. 2 demonstrates that A172 cells and U-118-MG cells possess abnormalities in several signal transduction pathways.

The factors produced by cancer tissues with activities to modulate surrounding microenvironment are evaluated by gene expression profiling. Important examples of interactions between cancer tissues and adjacent normal tissues include invasion, metastases, angiogenesis and immunosuppression. In both cell lines, as described in Fig. 2, genes encoding proteins responsible for adhesion, epithelial-mesenchymal transition, degradation of extracellular matrix, cell motility, implantation and angiogenesis are found to be strongly expressed.

**3. Perspectives**

The Project HOPE is characterized by several distinctive features to promote multiomics-based clinical practice.

First, the project is launched in the single institution under the crosstalk between clinicians and researchers, who are able to refer to all clinical information for evaluating genetic data of patients. This clinical setting encourages medical staff to implement individualized medicine for cancer patients. For instance, according to the criterion, most of the patients enrolled belong to stage II or III of the diseases, suggesting that around half of them will head into cancer recurrences. The Project HOPE provides medical staff with information on biological characteristics of the primary tumors, especially those of cancer-specific genetic alterations are able to be detected more precisely. These findings on glioblastoma cell lines indicate that the whole-exome sequencing and gene expression profiling in the Project HOPE are applicable for clinical evaluation for cancer tissues.

**Table 2**

<table>
<thead>
<tr>
<th>SNVs in signal transduction pathways</th>
<th>Gene expression of factors with activities to modulate microenvironment</th>
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</thead>
<tbody>
<tr>
<td><strong>Pathways</strong></td>
<td><strong>A172</strong></td>
</tr>
<tr>
<td>RTK &amp; RAS</td>
<td>EGFR*, ALK*, ETV4</td>
</tr>
<tr>
<td>GPCR</td>
<td>GPR124*</td>
</tr>
<tr>
<td>AKT</td>
<td>MTOR, PTEN, JAK3*</td>
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<tr>
<td>Wnt</td>
<td>CDH20, [ITGA10]</td>
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<tr>
<td>Hippo</td>
<td>NUMA1(A1827T)</td>
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<tr>
<td>TGFβ</td>
<td>NUMA1(A1638V)*</td>
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<tr>
<td>Warburg</td>
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<tr>
<td>Cell death/ NFKB</td>
<td>NUMA1</td>
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<tr>
<td>Notch</td>
<td>PTCH1</td>
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<td>Hedgehog</td>
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<td>FANCA, WRN</td>
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<tr>
<td>Cell cycle</td>
<td>TP53*, RB1, TAF1, [RNF213]</td>
</tr>
<tr>
<td>Others</td>
<td>SYNE1(K8079R), SYNE1(T2540M), THBS1*</td>
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</tbody>
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Fig. 2 Results of the model experiments using glioblastoma cell lines. In the left panel, single nucleotide variances (SNVs) in cancer-associated genes detected by the Project HOPE and those reported by COSMIC Cell Lines Projects (CCLP) in two glioblastoma cell lines, A172 and U-118-MG, are described. Unmarked genes are detected by both of the Project HOPE and the CCLP. Genes with asterisks are detected only by the Project HOPE, and genes in the square brackets are detected only by the CCLP. In the right panel, expression results of representative genes encoding proteins with activities to modulate surrounding microenvironment are demonstrated. In both cell lines, genes encoding proteins responsible for adhesion, epithelial-mesenchymal transition, degradation of extracellular matrix, cell motility, implantation and angiogenesis are found to be strongly expressed.
cancer driver genes and factors with activities to modulate surrounding microenvironment, which may be useful for the choice of molecular-targeting drugs at the time of cancer recurrences.

It is conceivable that multiomics-based medicine is still in the early stage in medical science, and the direction in which this type of medicine moves towards is not clear yet in routine clinical practice. However, new methodologies are developed very rapidly in this field. With regard to the Project HOPE, it is likely that specimens will be required for investigations by the whole-genome sequencing and epigenetic analyses in the near future. Since the project is a prospective study and enough-amount of tumor and normal tissue specimens can be available, it is preferable for investigators to analyze the specimens in storage, repeatedly, in order to obtain accurate results and newer information.

Secondly, the Project HOPE may afford an opportunity for medical staff to practice “preventive and presymptomatic medicine” or “Mibyo medicine” in Japanese, which was quoted from a Chinese book entitled “The Yellow Emperor’s Classic of Medicine,” written almost 20 centuries ago during the Han Dynasty (10). The concept still has a significance even in modern medicine, as it focuses on prevention of diseases by treating a patient “during preventive and presymptomatic stage” with sophisticated modern medical technologies.

Based on the genetic information of a patient’s diathesis from the results of blood cell analysis, medical staff are able to foresee the risk of acquiring diseases originated in such life-style habits as drinking alcohol and to utilize it for preventive medicine. Moreover, hereditary cancer syndromes or non-cancerous inheritable diseases can also be predicted and can be taken care of by preventive therapy, which should be beneficial for patient’s blood relatives as well. It is worth noting that appropriate medical intervention can reduce morbidity and mortality in these diseases. In the Project HOPE, non-cancerous inheritable diseases are evaluated according to ClinVar database; when sequencing results indicate the patient suffering from such morbidities, the observation will be disclosed to the patient with consultation by genetic counselors, according to guidance provided by American College of Medical Genetics and Genomics (6).

Thirdly, the project can provide learning experiences for medical staff and researchers in the field of multiomics-based analyses. The medical staff at the Shizuoka Cancer Center can treat patients after having learned about genetic changes of their cancers and diatheses. This does not only contribute to the improvement of cancer treatment, but also helps the medical staff to master genomic medicine. Meanwhile, the researchers in charge of analyses have chances to heighten the analytical accuracy from collating the analysis results with the clinical data. Accordingly, they can learn about how genetic changes are related to clinical conditions more in detail, and as a result, clinical tests based on genetic analyses will be more widely known and accepted in the society.

Lastly, the accomplishments of the Project HOPE can make important “seeds” for research and development in cancer medicine. We are expecting that new genetic changes of cancer tissues will be likely to result in developing new molecular-targeting drugs, biomarkers and tumor markers. The Shizuoka Cancer Center takes a lead in the Pharma Valley Initiatives, which aim to revitalize medical and health industries in collaboration with medical, research and industrial organizations. Collaboration between the Project HOPE and the Pharma Valley Initiatives may strengthen activities of research and development in this field.

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REFERENCES


