Next-Generation Sequencing Applications Complement Traditional Cytogenetic Methods

Genomic analysis of tumors provides comprehensive detection of genetic abnormalities.

Introduction

Accurate and efficient assessment of genetic variants in cancer research is important, yet not always straightforward. Various types of causative abnormalities have been identified, including chromosomal rearrangements, small insertions and deletions (indels), point mutations, and epigenetic alterations. Specific biomarkers may be highly represented in certain neoplasms calling for specific assays. But for many neoplasms, multiple genetic abnormalities can occur simultaneously, and heterogeneity adds further challenges to analysis.

While single-gene tests provide a quick answer in some cases, negative results may necessitate sequential testing that delays a conclusive result. Newer methods in genomic technologies provide more comprehensive testing by casting a wider net in search of causative variants. Also, they can complement single-gene assays by providing more specific information about the abnormality, or identifying additional genetic variants for monitoring later in the cycle of tumor progression.

When preliminary evidence indicates a cytogenetic abnormality as a likely cause of a neoplasm, fluorescence *in situ* hybridization (FISH) is often used in the first round of testing. FISH results can be obtained quickly, which is critical when information is needed to make informed decisions in a timely manner. However, negative FISH results necessitate sequential testing for alternate candidate targets, increasing the amount of lab work, and the total time required to yield meaningful answers. In these cases NGS complements FISH well. Performing both methods simultaneously could potentially reach desired answers more quickly.

Next-generation sequencing (NGS) is widely used by cancer researchers for oncology profiling, for its ability to identify multiple types of abnormalities, and sequence many genes simultaneously. NGS applications provide single nucleotide resolution with accuracy, sensitivity, and scalability. NGS works with DNA or RNA, depending on the questions being asked. These features increase the chances of obtaining answers within the first round of testing. NGS applications range from sequencing the entire genome to analyzing only a handful of genes. Targeted sequencing applications on a desktop sequencer enable the researcher to focus only on disease-related genes. Advantages of targeted sequencing include simplified analysis, speed, and higher sequencing depth on a selected set of genes, allowing detection of abnormalities present at a low level in heterogeneous samples.

This application spotlight reviews examples of neoplasms that pose challenges in sample analysis due to their variable characteristics, and describes how NGS offers flexibility that can complement cytogenetic assays. Together, these approaches provide a more comprehensive approach than either assay alone.

Myelodysplastic Syndromes (MDS)

MDS is a disease in which abnormal numbers of new blood cells are produced in the bone marrow. MDS progresses to acute myeloid leukemia (AML) in about 44% of cases.[1] The World Health Organization classifies MDS types according to phenotypes such as dysplasias, cytopenias, and anemias; preliminary characterization is often followed by cytogenetic or molecular analyses.[2-4] MDS has been associated with various types of abnormalities, including chromosomal rearrangements, single nucleotide variations, and epigenetic alterations.[4-7] No single method can detect all these abnormality types with efficiency, and, often, it is appropriate to use both NGS and FISH in a complementary manner to identify the relevant abnormality.

The most common chromosomal abnormalities found in MDS are the interstitial deletion of chromosome 5, monosomy 7, and trisomy 8. Cytogenetic studies are sufficient for identifying these abnormalities, but individually these genetic variations are found in only 10–20% of MDS cases.[3-5] Furthermore, studies have reported that at least 1 mutation occurs in 70–90% of MDS cases,[5-7] while 43% of cases have 2 or more abnormalities that are likely to be single nucleotide changes or small indels (Figure 1).[6] Because no specific abnormality is represented in a majority of MDS patients, sequential single-gene assays represent an inefficient approach to finding an abnormality in a given sample.

Labs that provide services for analyzing MDS samples have traditionally offered cytogenetic testing, immunohistochemistry, and flow cytometry. More recently, some labs have added molecular testing to improve risk stratification methods.[8] The National Comprehensive Cancer Network (NCCN) guidelines suggest molecular analysis for specific genes in various cases.[9] Some phenotypes, such as thrombocytosis, are often associated with *JAK2* mutations, and, therefore, molecular analysis of this gene is recommended. Varying levels of risk are associated with mutations in other genes. For patients that have a confirmed diagnosis of MDS, molecular analysis is recommended for *TP53*, *ASXL1*, *ETV6*, *RUNX1*, *SF3B1*, and *EZH2.*[9] Although these genes are among the most commonly associated with MDS, many more mutations have been found in MDS cases.[5-7] Fortunately, DNA sequencing panels can simultaneously assess many of the genes that are commonly associated with a given disease,[10] and increase the chances for identifying the relevant mutation in the first round of testing.

Molecular analyses have increasingly been used to identify abnormalities in MDS that cause changes in gene expression levels. For example, mutations in spliceosome-related genes are found in about 45% of MDS cases.[6] Epigenetic changes are more abundant in high-risk cases of MDS, with 70% showing aberrant DNA methylation in genes related to cell growth.[6] RNA sequencing with NGS provides

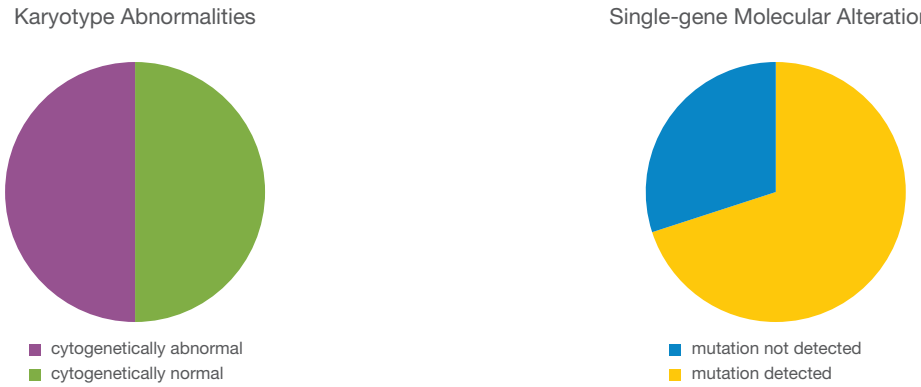

Figure 1: Frequency of genetic aberration types found in MDS—Karyotype abnormalities are detected in approximately 50% of MDS cases, while single-gene molecular alterations are detected in approximately 70% of MDS.[6]

information on the sequence of a gene, but also its activity within a tumor. Abnormal gene expression of tumor-related genes can result from mutations in the coding region, mutations in the regulatory regions, aberrations in the spliceosome factors, or by epigenetic alterations. Sometimes, using RNA sequencing as an early method of analysis can directly confirm the mechanism of expression change, and in some cases, can give preliminary indications of the cause of expression variations. This preliminary evidence can aid in the selection of more specific assays to confirm the exact mechanism, and to rule out other causes.

Sarcomas

Approximately one-third of all sarcomas are associated with chromosomal translocations that lead to fusion gene products and adversely affect cell cycle control.[11] Because gene fusions usually result from large chromosomal rearrangements, FISH can be an efficient method to identify the causative genetic abnormality. However, in any tumor for which the causative abnormality is not known, NGS may also be an appropriate method for first-round analysis, even when there is already a likely candidate.

Classification of soft tissue sarcomas with immunohistochemistry and morphology can sometimes point to subtypes in which specific translocations are prevalent, but does not definitively demonstrate the presence of these fusions. Although FISH can identify gene fusions, the number of described fusion genes has increased with the advent of molecular analyses, indicating that a comprehensive approach to characterizing tumors is appropriate in many cases. More than 1000 gene fusions have been described in humans, with > 94 of these fusions identified in sarcomas distributed among 30 distinct subtypes.[12]

RNA sequencing with NGS is a valuable tool for the analysis of tumors in which gene fusions are common, but where the number of potential fusions is too numerous to cover efficiently with a single multiplexed PCR assay. Targeted RNA sequencing panels can simultaneously cover hundreds of fusion-associated genes, increasing the likelihood of identifying an abnormality in the first try, and decreasing the chance of reflexive testing. By providing single nucleotide resolution, sequencing enables localization of chromosomal breakpoints, which may help in downstream decision making. Prior knowledge of specific translocations is not required for RNA sequencing applications to discover novel fusion partners.

In addition to gene fusions, RNA sequencing can detect aberrations that many DNA tests would miss, such as abnormalities that lead to altered gene expression. For example, multiple sarcoma types exhibit mutations in promoter regions of the TERT gene.[13,14] RNA sequencing can detect deregulation of genes that affect the cell cycle, regardless of whether the event results from DNA mutations in the coding region, DNA mutations outside the coding region, chromosomal translocations, or epigenetic alterations. As molecular assays with wide coverage are applied to these heterogeneous diseases, the continued possibility exists for discovering new mechanisms of disease progression.

Conclusion

For many types of neoplasms, multiple types of genetic abnormalities are commonly found, and a single-gene assay is not always the best method for characterizing the disease. Using NGS in a complementary role alongside FISH is a comprehensive approach that can reveal valuable information on novel fusion partners and emerging biomarkers of interest. Although FISH is a rapid method for identifying relatively large chromosomal rearrangements 1 target at a time, casting a wider net that covers numerous candidate genes can be a more efficient approach to identify biomarkers that facilitate informed decisions.

With a broad range of NGS applications available, Illumina provides researchers with flexible, accurate, and reliable tools for analyzing the genome or transcriptome. NGS panels can assess tens to thousands of genes simultaneously, and can be customized to fit customer needs. Targeted DNA sequencing panels can assess point mutations and small indels in most genes that are associated with a given type of disease. Targeted RNA sequencing can simultaneously assess single nucleotide variations, alterations in gene expression, and breakpoint resolution in gene fusions with both common and novel partners. NGS relies upon state-of-the-art equipment, yet is provided with simple analysis software that performs data analysis at the click of a button, providing access to even inexperienced users. Through partnerships with leading oncology experts and collaboration with national and international cancer organizations, Illumina continues to expand the portfolio of cancer focused research solutions.

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