Utilization of Genomics and Functional Genomics to Inform Clinical Decisions in IR

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ABBREVIATIONS

lncRNA = long noncoding RNA, LRT = locoregional therapy, miRNA = microRNA, RNA-Seq = RNA sequencing, WES = whole-exome sequencing, WGS = whole-genome sequencing

The age of precision medicine has ushered in greater appreciation of the influence of genomics and functional genomic variation on fundamental tumor biology and their role in predicting patient prognosis, treatment allocation, and therapeutic response (1). Genetic analyses offer the capability to provide insights into primary biologic mechanisms not discernible using current conventional histologic and radiologic techniques. Owing to the key role interventional radiologists play in image-guided tumor biopsy and administration of targeted therapies and locoregional therapies (LRTs), a firm understanding of molecular analyses used for genomic profiling in personalized medicine is critical, particularly considering the recognized gaps in knowledge relating to the fundamental factors contributing to LRT effectiveness and disease relapse. As genomic and functional genomic analyses have the potential to identify intrinsic tumor biology underlying differential treatment responses, it is imperative that interventional radiologists understand the role these molecular analyses can play. This review provides an overview of genomic analytic approaches, with attention to their potential utility in contemporary interventional radiology (IR) clinical practice.

GENETIC CHANGES IN CANCER

Cancer comprises a constellation of diseases in which malignant cell growth is driven by somatic genetic mutations that can result from environmental factors or inherited germline genetic variation. Genetic mutations driving tumorigenesis include single nucleotide polymorphisms, small insertions and deletions, insertion or deletion of entire gene regions (copy number variations), and chromosomal translocations resulting in altered gene regulation or chimeric genes. Not all somatic mutations trigger oncogenesis; mutations involving genes or regulatory regions (ie, promoters) resulting in malignancy are referred to as driver mutations. In addition to driver mutations, so-called passenger mutations—which have no significant effect on tumor biology—accumulate over time, making it difficult to identify phenotypically important changes. Driver mutations can affect gene expression and epigenetic patterns—environmentally influenced alterations that change DNA accessibility and chromatin structure—resulting in altered gene regulation without affecting the DNA sequence. Epigenetic mechanisms are commonly distorted in cancer cells, with DNA methylation, long noncoding RNA (lncRNA), and microRNA (miRNA) representing the most heavily studied and promising epigenetic mechanisms for use as clinical biomarkers. As alterations in epigenetic and gene expression patterns—together referred to as functional genomic alterations—result in phenotypic changes that influence intrinsic tumor biology, use of genomic information in combination with functional genomic profiling is critical to help distinguish driver and passenger mutations.

GENOMIC ANALYTIC APPROACHES

Tissue Sample Procurement

Although determining the optimal combination of sequencing strategies required warrants careful consideration, of primary concern is the quality of available tissue samples. Clinical biopsy specimens are typically prepared as formalin-fixed paraffin-embedded samples for histologic interpretation; however, this processing results in sample degradation and introduction of sequencing artifacts (sequence changes not present in the original sample) into nucleic acids. Nucleic acid quality is most severely affected

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if a sample is formalin fixed for > 24 hours, which results in variable DNA and RNA quality and low yields unsuitable for genomic analyses. For these reasons, freshly collected specimens flash frozen in liquid nitrogen within 10 minutes and stored at −80°C are ideal for preserving DNA methylation patterns and RNA integrity. Ideally, multiple tumor biopsy specimens should be obtained to ensure the presence of adequate amounts of viable malignant cells reflecting the tumor heterogeneity. Care should also be taken to separate tumor and benign regions before freezing or before extraction from formalin-fixed paraffin-embedded samples, although specimens containing as little as 10% tumor cells can still be used to identify genetic variation. From a technical standpoint, use of a new biopsy device for each needle pass should be considered to avoid contamination across tissue samples.

In addition to tumor biopsy, liquid biopsy (ie, sampling of blood, plasma, urine, or other bodily fluid) is emerging as a promising noninvasive method to assess tumor biology through detection of circulating tumor cells, metabolites, and cell-free DNA and/or RNA originating from dead tumor cells. Reduced risk, pain, and cost represent significant potential advantages of liquid over tumor biopsies. As with tumor biopsy specimens, liquid biopsy samples are ideally flash frozen within 10 minutes of collection followed by storage at −80°C until processing. As this is not always feasible in clinical settings, commercial products are available to reduce RNA degradation for tissue (eg, RNA*later*; Qiagen, Hilden, Germany) and blood (eg, Tempus Blood RNA Tubes; Thermo Fisher Scientific, Waltham, Massachusetts) samples that cannot be immediately frozen.

Genomic Variation Analysis

A variety of techniques are available that leverage sequencing technologies to identify genomic variation or quantify gene expression and epigenetic mechanisms. Genomic variation analysis fundamentally relies on nucleic acid (DNA and/or RNA) sequencing techniques, which can focus either on one or a few short sequences at a time (ie, Sanger sequencing) to investigate a specific genomic region or on rapid sequencing of potentially millions of fragments (ie, Illumina sequencing; Illumina, Inc, San Diego, California), also known as next-generation sequencing, to investigate genome-wide variation as a single assay. There are a number of genome-wide and targeted approaches that can be used depending on the type of variation being explored (Fig 1). For genomic variation, the 2 most commonly used approaches are whole-genome sequencing (WGS) and whole-exome sequencing (WES). WGS consists of fragmenting and sequencing genomic DNA to profile the entire genome. Although WGS is ideal for identifying genomic variation (eg, single nucleotide polymorphisms, small insertions and deletions, copy number variations) across the entire genome, producing the sequencing depth (number of reads covering each region) required to identify somatic variation present in a minority of tumor cells can be cost prohibitive. To reduce these costs, WES approaches utilizing probes with sequences complementary to known gene coding regions are used to target gene-coding regions for sequencing. As gene-coding regions represent approximately 2% of the human genome, WES allows for investigation of all known gene-coding regions, while significantly reducing sequencing costs. However, as this approach targets only known coding regions, genomic variation in noncoding regions (eg, promoters or enhancers) that can have significant effects on gene regulation will be missed.

DNA Methylation Analysis

Although profiling genomic variation can identify potential driver mutations, functional genomic variation can provide insights into the effects of these mutations on epigenetic and gene expression patterns directly affecting tumor biologic phenotypes. One of the most well studied and promising epigenetic mechanisms for prediction of patient prognosis and treatment responses is DNA methylation. DNA methylation refers to the addition of a methyl group to cytosine bases throughout the genome. This typically occurs at cytosine bases that are followed by a guanine nucleotide along the 5′ to 3′ direction of a linear DNA sequence (CpG sites), with low methylation in promoters and transcription start sites associated with gene expression. DNA methylation is commonly altered in cancer cells resulting in aberrant gene expression and uncontrolled cellular proliferation. Methylated and unmethylated cytosines can be differentiated through treatment of DNA with sodium bisulfite followed by sequencing (bisulfite sequencing) (Fig 1). During the sodium bisulfite treatment, unmethylated cytosines are converted to uracil, while methylated cytosines remain unchanged. The resulting uracil bases are sequenced as thymines, and the ratio of cytosine base calls to cytosine plus thymine base calls represents the percent methylation at a given genomic site. As with genomic sequencing, 2 main approaches are used to profile DNA methylation patterns: whole-genome bisulfite sequencing, which profiles all cytosine bases in the genome, and reduced representation bisulfite sequencing, which uses restriction enzymes and size selection to target CpG-rich regions enriched for promoter regions and transcription start sites.

RNA-Based Quantification Analysis

The final layer of functional genomic variation affected by the aforementioned genomic and epigenomic variations is RNA expression. Three types of RNA of interest for predicting clinical phenotypes are messenger RNA, which encodes genes to be translated into proteins, and noncoding lncRNA and miRNA, which regulate gene expression at both the transcriptional and the translational levels. Standard RNA sequencing (RNA-Seq) approaches allow for identification and quantification of lncRNAs, genes, splice variants, and fusion genes affecting tumor biology (Fig 1). miRNA expression is measured in the same manner but with an additional pre-processing step to isolate small RNAs before sequencing (small RNA-Seq) (Fig 1). RNA-Seq can also be combined with probes with sequences