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Fusion genes in solid tumors: the possibilities and the pitfalls

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Though recurrent fusion genes were initially identified in hematopoietic neoplasms, these genetic events were subsequently found in solid tumors, including sarcomas, carcinomas, brain tumors, and benign tumors [1]. Fusion genes can be formed by a variety of chromosomal breakage and rejoining events, including translocations, inversions, deletions, and duplications. The common feature of these events is the joining of two noncontiguous genetic loci, thereby juxtaposing regulatory and/or protein-coding elements from the two loci. In comparison to the wild-type genes, the resulting fusion genes express RNA and protein products with altered regulation and/or structure.

The relationship of fusion genes to histopathologic categories of solid tumors is complex. There are multiple examples of fusion genes that only occur in a single category and more over in most cases of that category [2]. However, other fusions are characteristic events in several different categories, such as ETV6-NTRK3, which is found in infantile fibrosarcoma, secretory breast carcinoma, mammary analogue secretory carcinoma, and acute myeloid leukemia [3]. Finally, there are fusions (such as those involving ALK or BRAF) that occur in small subsets of one or more tumor categories [4]. Some of these subsets correspond to biologically distinct, yet histologically inapparent categories, whereas other fusion subsets represent just one of several genetic strategies to deregulate growth signaling in the associated lineage.

There is also significant genetic heterogeneity associated with certain fusion genes [5]. Though some rearrangement breakpoints consistently involve single introns and produce an invariant RNA and protein product, breakpoints of other fusions rearrange one of multiple introns and result in a variable fusion product. In addition, functionally related genes can be used as alternative partners and result in variant fusions within a tumor category. For example, translocation breakpoints in Ewing's sarcoma occur in EWSR1 introns 7 through

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Declaration of interest

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10 and FLI1 introns 3 through 8 to produce a variably sized EWSR1-FLI1 transcript and protein. Furthermore, EWSR1 can be replaced by the related RNA-binding protein FUS, and FLI1 can be replaced by four other ETS-family transcription factors in Ewing's sarcoma-associated fusions.

In tumors and nonneoplastic cells, RNA fusions may also be generated without DNA rearrangement [6]. Read-through fusion transcripts are formed by transcription and splicing of two adjacent genes. Furthermore, exons from two distinct primary transcripts can be joined in the process of trans-splicing. To further complicate matters, a few recurrent fusion transcripts associated with chromosomal translocations in solid tumors are also expressed by trans-splicing in related nonneoplastic cells.

To utilize these novel molecular markers in clinical practice, molecular diagnostic methods were developed to assay for these fusions in tissue samples. Two common approaches are fusion mRNA detection by reverse transcription (RT)-PCR and fusion gene detection by fluorescence *in situ* hybridization (FISH) [7]. Each assay only requires small amounts of tissue and can be applied to formalin-fixed paraffin-embedded samples. RT-PCR is distinguished by its higher analytical sensitivity, which makes this method useful in detecting submicroscopic disease. However, RT-PCR requires prior knowledge of both fusion partners and is less effective in situations with significant potential heterogeneity. In contrast, the FISH splitting assay has lower analytical sensitivity but detects rearrangement of one partner without knowledge of the second partner and thus is more applicable to settings with high potential heterogeneity.

As an alternative approach, immunohistochemistry (IHC) is a commonly available technology and permits direct visualization of the fusion protein in tumor cells [8]. The optimal IHC approach for fusion testing utilizes antibodies to N-terminal and C-terminal portions of a wild-type protein and thereby detects fusion proteins based on the finding that only part of the wild-type protein is expressed. However, most IHC approaches rely on one antibody, which does not distinguish between the fusion or wild-type protein, thereby lowering the diagnostic specificity of this assay. For those situations in which the wild-type protein is infrequently expressed, IHC provides a useful adjunct test.

In recent years, next-generation sequencing approaches were developed to simultaneously screen for many different fusions and ultimately to enable agnostic screening for any fusion [9]. Though exome sequencing is frequently used to detect small mutations, this technology is not generally useful for fusion detection, since most breakpoints occur within introns or other noncoding areas. In contrast, genome or transcriptome sequencing readily detects fusion genes. To avoid the high coverage and accompanying cost needed for global analyses, smaller focused sequencing panels were developed to detect varying numbers of fusion genes. As sequencing costs decrease in coming years, whole transcriptome or whole genome sequencing will become more feasible as routine clinical assays. Though these global strategies do not require any prior knowledge of which fusion should be assayed, these approaches will detect all fusions within the sample, regardless of functional or clinical significance [10]. In particular, whole transcriptome sequencing detects all fusion mRNAs, including read-through or trans-spliced transcripts. Whole genome sequencing identifies all

rearranged fragments, which can be numerous in tumors with genomic instability and often have no functional consequences. Clinical translation of these global approaches will require increased bioinformatic capabilities and improved methods with effective filters for nonfunctional and insignificant bystander events.

These molecular diagnostic assays are applicable to a number of clinical situations. As described earlier, some fusions have high diagnostic sensitivity and specificity. As an example, for the diagnostic quandary of the undifferentiated small round cell tumor, a panel of fusion assays can help to rule in or out specific tumor categories, such as Ewing's sarcoma, rhabdomyosarcoma, and desmoplastic small round cell tumor, within the differential diagnosis [11]. In other cases in which the pathologist has narrowed the differential diagnosis to a probable category, the detection of a fusion gene lends increased confidence to the final diagnosis. For some tumors, it is probable that the finding of a characteristic fusion will become a requirement for entry onto related clinical trials.

The utility of fusion genes as markers in differential diagnosis must be qualified based on the association of some fusion genes with multiple distinct histopathologic entities. As described earlier, some fusions such as ETV6-NTRK3 fusion can occur and are oncogenic in mesenchymal, epithelial, and hematopoietic lineages [3]. Furthermore, the finding of the EWSR1-ATF1 and EWSR1-CREB1 fusions in multiple clinically distinct sarcoma categories emphasizes that neoplastic phenotype depends on the cell of origin as well as the genetic event [12]. Therefore, the finding of a characteristic fusion gene in a seemingly incompatible histopathologic picture may not provide a definitive diagnostic answer until the relative importance of the fusion gene and histopathologic pattern is unraveled [13].

Molecular detection of fusion genes is also useful in outcome prediction and risk assessment. Depending on the clinical scenario, a difference in outcome may be predicted by the presence or absence of a specific fusion, the presence of one of several variants of a fusion, or the presence of one of a heterogeneous group of unrelated fusions [14,15]. Based on these outcome associations, fusion status can be utilized with other clinical parameters to establish a risk classification system that assigns patients to different treatment regimens. In addition to identifying fusions in macroscopic tumors, assays such as RT-PCR can be applied to detect submicroscopic involvement in sites such as bone marrow and lymph nodes [16]. In this way, fusion analysis may be used to more accurately stage disease spread and potentially improve risk assessment and treatment planning. In addition, these PCR-based assays can also be used to for screen for evidence of fusion-associated cancers in body fluids, such as blood and urine, to help with initial diagnosis or to detect early relapse [17].

Finally, identification of a fusion in a tumor can indicate susceptibility to specific therapy. Some fusion proteins are direct therapeutic targets, such as EML4-ALK in lung adenocarcinoma, which is inhibited by crizotinib [18]. In other cases, the fusion gene product interacts with a targetable receptor; for example, in dermatofibrosarcoma protuberans, the COL1A1-PDGFB fusion protein is processed to give high levels of PDGFB, which then binds to and activates the PDGF receptor, a known target of imatinib [19]. In a third treatment scenario, since numerous fusion proteins impact directly or indirectly on transcription, an upregulated downstream product may be a target for inhibitory

therapy, as proposed for the MET protein, which is upregulated by fusion proteins in several sarcoma categories [20].

In summary, fusion genes in solid tumors constitute an expanding armamentarium of molecular markers. These markers provide clinically useful information that impacts on the diagnosis, prognosis, or therapy of the associated tumors. As the biological and clinical characteristics of each fusion are unique, there are few definitive rules for applying these fusions to patient care. The biological, genetic, and clinical information for each fusion and tumor category must be carefully assessed to develop appropriate approaches for using these molecular markers in patient management.

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