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Minireview

Mutation-associated fusion cancer genes in solid tumors

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Abstract

Chromosomal translocations and fusion oncogenes serve as the ultimate biomarker for clinicians as they show specificity for distinct histopathologic malignancies while simultaneously encoding an etiologic mutation and a therapeutic target. Previously considered a minor mutational event in epithelial solid tumors, new methodologies that do not rely on the detection of macroscopic cytogenetic alterations, as well as access to large series of annotated clinical material, are expanding the inventory of recurrent fusion oncogenes in both common and rare solid epithelial tumors. Unexpectedly, related assays are also revealing a high number of tandem or chimeric transcripts in normal tissues including, in one provocative case, a template for a known fusion oncogene. These observations may force us to reassess long-held views on the definition of a gene. They also raise the possibility that some rearrangements might represent constitutive forms of a physiological chimeric transcript. Defining the chimeric transcriptome in both health (transcription-induced chimerism and intergenic splicing) and disease (mutation-associated fusion oncogenes) will play an increasingly important role in the diagnosis, prognosis, and therapy of patients with cancer. [Mol Cancer Ther 2009;8(6):1399–408]

Introduction

Cancer genes are best defined by the presence of recurrent tumor specific DNA alterations, and the Cancer Gene Census of the Welcome Trust Sanger Institute has recently tabulated a working list of 384 validated genes representing more than 1.5% of all transcribed human genes.[1] Although these mutations can include gene amplification, point mutations, and small insertions or deletions, recurrent chromosomal translocations and fusion oncogenes are the most frequent type of somatic DNA alteration detected in 282 of the 384 validated cancer genes. In addition, other databases have cataloged an even greater number of fusion gene partners (1). The clinical impact of these types of gene rearrangement events on translational research of adult solid tumors, however, has been limited because these events have been overwhelmingly linked to hematological malignancies or uncommon soft tissue sarcomas. Renewed interest in this topic has now been stimulated by a growing number of cryptic fusion oncogenes that have been unexpectedly associated with a subset of common adult cancers, such as adenocarcinomas of the lung, prostate, kidney, and breast (2–5), as well as uncommon solid tumors, such as malignant salivary gland cancers (6). Therefore, as predicted by Mitelman and colleagues (7), the list of gain-of-function fusion cancer genes in solid tumors is certain to increase as more epithelial tumor samples become available for testing using new methodologies that do not necessarily rely on the ability to detect a cytogenetic abnormality (Table 1). Although the finding of numerous low-frequency somatic mutational events in human cancer poses challenges for clinical therapeutics, the elucidation of signaling pathways resulting from these new discoveries and the ultimate organization of this data into discrete interconnecting cancer gene pathways will greatly improve our ability to classify these tumors into highly homogenous groups for diagnosis and prognosis, and may ultimately offer the best chance for success with new targeted therapies.

Fusion Gene Events Provided a Framework for the Mutational Theory of Cancer

There are several reasons why fusion cancer genes have captured the imagination of cancer biologists and clinicians since the first description of the Philadelphia chromosome in 1960 (8). First, the identification of the recurrent t(9;22) chromosomal translocation in chronic myelogenous leukemia (CML) provided a tangible framework for the gene mutational theory of cancer, initially proposed by Boveri in 1902 as an aberrant tumor-specific "chromosomal aggregation" during mitosis (9). Subsequent refinements in cell culture and cytogenetic techniques allowed investigators to catalog an increasing number of chromosomal alterations, particularly in hematological cancers (10). However, it was

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[1] The data were obtained from the Wellcome Trust Sanger Institute Cancer Genome Project web site, http://www.sanger.ac.uk/genetics/CGP.

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Table 1. Fusion oncogenes detected in nonsarcoma solid tumors

Nonsarcoma Solid Tumors	Fusion Oncogenes
Aggressive midline carcinoma	BRD4-NUT, BRD3-NUT
Astrocytoma	KIAA1549-BRAF, FIG/GOPC-ROS1
Secretory breast carcinoma	ETV6-NTRK3
Breast cancer*	BCAS4-BCAS3, TBL1XR1-RGS17, ODZ4-NRG1
Kidney carcinoma†	MALAT1-TFEB, ASPSCR1-TFE3, PRCC-TFE3, CLTC-TFE3, NONO-TFE3, SFPQ-TFE3
Mesoblastic nephroma‡	ETV6-NTRK3
Lung adenocarcinoma	EML4-ALK
Melanoma of soft parts‡	EWSR1-ATF1
Meningioma‡	MN1-ETV6
Salivary gland:	
pleomorphic adenoma	CTNNB1-PLAG1, LIFR-PLAG1, TCEA1-PLAG1, FGFR1-PLAG1, CHCHD7-PLAG1, HMGA2-FHIT, HMGA2-NFIB
mucoepidermoid cancer	CRTC1-MAML2, CRCT3-MAML2 EWSR1-POUF5F1
Prostate carcinoma	TMPRSS2-ERG, TMPRSS2-ETV1, TMPRSS2-ETV4, TMPRSS2-ETV5, HNRNPA2B1-ETV1, HERV-K-ETV1, C15ORF21-ETV1,SLC45A3-ETV1, SLC45A3-ETV5, SLC45A3-ELK4, KLK2-ETV4, CANT1-ETV4
Thyroid carcinoma	RET-PTC1/CCDC6, RET-PTC2/PRKAR1A, RET-PTC3,4/NCOA4, RET-PTC5/GOLGA5, RET-PTC6/TRIM24, RET-PTC7/TRIM33, RET-PTC8/KTN1, RET-PTC9/RFG9, RET-PCM1, TFG-NTRK1, TPM3-NTRK1, TPR-NTRK1 RET-D10S170, ELKS-RET, HOOKS3-Ret, RFP-RET, AKAP9-BRAF, PAX8-PPARG

*Detected in breast cancer cell lines.
†Often pediatric cases and young adults.
‡Tumors exhibit mixed sarcomatous features.

the reductionist discovery in 1983 that the cellular homolog of a tumor virus oncogene was specifically targeted by the recurrent translocations in CML (c-Abl gene) (refs. 11–13) and in Burkitt's lymphoma (c-myc gene) (ref. 14) that the mutational and clonal expansion theory of cancer was firmly established. A second important feature of fusion oncogenes for clinicians was their striking association with a specific clinico-pathologic tumor entity. For example, although recurrent somatic point mutations of selected viral oncogene homologs were also identified in many human tumors (15, 16), the presence of these mutations did not usually confer the same specificity for a single histologic entity as seen with the unique promoter swapping and ectopic gene expression that characterizes chromosomal translocations (see below). Finally, recurrent, mutation-associated fusion oncogenes are, by definition, gain-of-function events and, until efficient gene replacement strategies are developed to counter loss-of-function mutations in cancer, these activated cancer genes offer the best potential targets for clinical therapeutics. For example, the use of all-*trans*-retinoic acid for acute promyelocytic leukemia carrying a t(15;17) rearrangement (17) or of imatinib therapy for CML carrying the Philadelphia chromosome (18) have reinvigorated the Ehrlich concept of targeted therapy in cancer. Therefore, there is optimism that this approach will show continued success with both specificity and predictive power in which variations in gene partners can have a major impact on the efficacy of targeted therapy (19) and, conversely, a dramatic response to a specific targeted agent can serve as a surrogate to identify an otherwise cryptic gene rearrangement (20).

Traditional Cytogenetic-Based Methods for Fusion Gene Detection

A common method to identify fusion cancer genes has been guided by the ability to detect a recurrent chromosomal alteration using standard or high resolution cytogenetics, such as spectral karyotyping. This can allow for a positional strategy such as fluorescent *in situ* hybridization (FISH) using dual color probes to map breakpoints on the derivative chromosomes (21). The selection of candidate FISH probes has been streamlined by updated curations of the human genome sequence with correction of contig and probe mapping errors that plagued earlier investigations such that breakpoints can now be identified rapidly. Accordingly, the rate limiting steps for these types of studies is the ability to both detect a recurrent cytogenetic rearrangement as well as the availability of a sufficient supply of tumor cells for serial testing. In fact, Mitelman and colleagues proposed in 2004 that the enrichment of chromosomal translocations reported in hematological tumors may be partly attributed to the relative ease of analysis in leukemias and, conversely, to the much greater difficulty in both tumor collection and cytogenetic analysis in solid tumors (7). For example, a recurrent reciprocal chromosomal alteration

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involving chromosomes 6q and 9p has been reported for several years in adenoid cystic carcinoma of the salivary glands (22–24), however, efforts to identify this breakpoint have been stymied perhaps because of limited biopsy material and to the unavailability of a continuous xenograft or tumor cell line source. Alternate methods for cancer gene detection that do not rely on sequential cytogenetic and positional cloning analyses, therefore, might be particularly fruitful for the future detection of fusion cancer genes in solid tumors. In fact, variations of older assays, as well as novel bioinformatic approaches that are independent of cytogenetic data, have been recently applied to the discovery of unexpected fusion cancer genes for both solid and hematological tumors.

Noncytogenetic-Based Methods for Fusion Gene Detection

Modified NIH 3T3 Transformation Foci Assay

For example, to discover new activated forms of oncogenes, investigators recently employed a modification of the classic NIH 3T3 transformation assay (25, 26) by generating an expression cDNA library enriched for full-length transcripts using RNA extracted from a patient with lung adenocarcinoma (3). NIH 3T3 cells were infected with retroviral-packaged cDNA, transformed NIH 3T3 foci were harvested at 2 weeks in the absence of antibiotic selection, and insert cDNA recovered from the foci using flanking plasmid primers for PCR amplification followed by nucleotide sequencing. The detection of a chimeric EML4-ALK fusion cDNA as the transforming event was unexpected but has now been confirmed by investigators with a frequency in lung cancer of approximately 3% to 7% (27). In addition, multiple variant chimeric isoforms have been isolated (28), and intensive preclinical and clinical investigations are underway to explore the efficacy of small molecule ALK inhibitors for these cases (29). These observations have also reinforced three important points about fusion oncogenes in solid tumors: (1) the specificity of fusion partners for distinct histopathologic entities in which ALK gene partners differ between anaplastic large cell lymphoma versus lung adenocarcinoma, (2) that many newly discovered fusion oncogenes in selected common adult cancers, such as lung cancer, will be low frequency events resembling the accumulating data on low frequency somatic point mutations from global resequencing efforts (30), and (3) that future etiologic gene rearrangements will often be detected in the absence of a macroscopic cytogenetic alteration. In fact, small or large intrachromosomal deletion events with or without inversions, such as was observed with the EML4-ALK fusion on chromosome 2p in lung cancer, are altering our prior assumptions that recurrent hemizygous deletions must harbor a loss-of-function event. However, the most important recurring theme for fusion oncogenes in solid tumors is that these recently identified gain-of-function mutational events, such as EML4-ALK, will offer the best chance for meaningful therapeutic strategies for a growing number of patients with otherwise incurable disease.

Monitor for Outlier Clinical Response to Targeted Therapy

An uncommon, but nonetheless illuminating, story for the discovery of somatic gain-of-function mutations was the astute observation by a group of physicians in private practice of a dramatic response to imatinib given "off-label" to a patient with rapidly progressive primary hypereosinophilic syndrome (31). This observation was followed by several confirmatory clinical trials and also triggered the reasonable hypothesis that clonal activation of an imatinib-response protein kinase may underlie this disease (20). 5′ rapid amplification of cDNA ends (RACE) experiments anchored from a candidate tyrosine kinase gene revealed a novel FIP1L-PDGFRA chimeric transcript that again arose from an interstitial hemizygous deletion aberrantly linking two genes normally located about 800 (kilobases) kb apart on chromosome 4 (20), which would not be detectable by standard cytogenetics. A similar outlier response to the use of gefitinib therapy in a patient with lung cancer was also credited with stimulating the search and eventual detection of etiologic-activating EGFR mutations in those responsive lung cancer patients (32). Genetic scrutiny of tumor samples from patients with similar outlier therapeutic responses, therefore, may continue to yield occasional discoveries of activating fusion and nonfusion cancer gene mutations.

Monitor for Outlier Gene Expression Patterns

Because gene fusion events are associated with ectopic and often elevated steady-state expression of the chimeric mRNA, the search for genes that are unexpectedly expressed at higher levels in discrete subsets of tumor samples may identify candidate gain-of-function cancer genes. Investigators coined the term "cancer profile outlier analysis" (COPA) and applied this bioinformatics approach to the Oncomine public database and identified candidate outlier genes that led directly to the identification of recurrent fusions between the 5′ untranslated region of TMPRSS2 and either the ERG or ETV1 oncogene in prostate adenocarcinoma (3). This was a landmark observation as (1) it was the first demonstration of a high frequency fusion oncogene event (40%-80%) in a common adult carcinoma (33) and (2) identified a biological mechanism for tumorigenesis with the androgen-dependent TMPRSS2 promoter driving the ectopic expression of ETS cancer gene members. The immediate hope is that knowing the status of specific gene fusion partners in prostate cancer will confer sufficient information on the expected natural history to allow a better triage of therapy for both early and late-stage disease. There is no better disease to test this hypothesis than in early-stage prostate cancer in which both patients and physicians have struggled to weigh the risks and benefits of definitive treatment with either surgery or radiation therapy versus aggressive surveillance (34). In addition, global gene expression analyses will continue to play an important role in new cancer gene discovery, as recently reported for the identification of SPINK1 overexpression as an alternate target in fusion negative prostate cancer samples (35).

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Massively Parallel Paired-End Sequencing

Another strategy to identify fusion cancer genes takes advantage of new sequencing technology platforms to obtain short reads from both ends of genomic DNA fragments obtained from tumor samples (36–38). Discrepancies in the alignments of the paired sequence reads, as compared with the predicted reference human genome, can then be scored and investigated. Although such discrepancies may arise from chromosomal rearrangements leading to the identification of new fusion transcripts, many alterations represented internal deletions, insertions, duplications, and other events that were often triggered by either repeat elements or regions of discrete copy number variation. In addition, because this analysis was not guided by functional data, several of the rearrangements detected were often predicted to encode "loss-of-function" phenotypes, including an out-of-frame fusion between the CACNA2D4-WDR43 genes (38). A benefit of this approach, however, is the use of genomic rather than cDNA templates that offers the potential to capture chromosomal rearrangements that have breakpoints beyond transcriptional start site, which would not be detected by reverse transcriptase (RT)-PCR or RACE strategies that rely on chimeric transcripts. With continued improvements in the length and accuracy of base-call reads, these applications should continue to advance the scope of fusion gene analysis in solid tumors.

Transcriptome Sequencing

Finally, although some chimeric transcripts are censored in the public databases, surveys of the public mRNA and EST databases is another bioinformatic strategy that can confirm previously known fusion genes as well as discover new candidates. For example, one study identified approximately 60 previously reported fusion genes and 20 new putative chimeric genes (39) providing validation for this approach. Ultimately, high throughput sequencing with deep surveys of the transcriptome, currently proposed as an improved method over array based platforms to study the diversity of alternative exon splicing (40), may offer the best strategy to fully annotate the chimeric transcriptome of cancer. In fact, a recent study using both long- and short-read sequencing platforms showed the ability to rediscover previously identified (BCR-ABL and TMPRSS2-ERG) as well as novel fusion events (SLC45A3-ELK4) and other chimeric transcripts in defined tumor cell lines (41).

The Chimeric Transcriptome in Health and Disease

There are two broad classes of fusion transcripts: (1) the recently rediscovered entity called "transcription-induced chimerism" that can occur from "run-on" transcription with a retained intervening sequence or intergenic splicing and *cis*- and *trans*-splicing, which may have both a physiological and cancer gene role (42–46) and (2) the constitutive, mutation-associated fusion oncogene that results in ectopic gene expression with either intact or disrupted open reading frames of the corresponding reciprocal gene partners (Fig. 1).

Transcription-Induced or *cis*- and *trans*-Splicing Chimerism

Transcription-induced chimerism has been noted in selected mammalian genes over the past decade (47) and is increasingly recognized as a physiologic event in normal tissues that may also confer hypothetical protumorigenic signals via either gain-of-function or loss-of-function effects (42–44). Although intergenic splicing events are frequently detected in the genome with overexpression of some tandem transcripts preferentially in tumor versus normal tissues, the biological role of these events is still unknown as there may not always be a strong selection to maintain the open reading frame or express a chimeric protein product (42, 44, 45). Remarkably, a comprehensive analysis of the 5′ transcription start site of 399 annotated protein coding genes (ENCODE pilot project) using pooled 5′ RACE products to hybridize with a high density genome tiling array showed that approximately 50% of the genes had distal 5′ exons that often spanned adjacent genes ranging more than a distance of up to 200 kb (48). These observations will also raise important questions about the definition of a gene. Of particular interest was the observation that intergenic splicing between the MDS1 and EVI1 genes on chromosome 3q in normal tissues marked a breakpoint region where this loci was occasionally targeted for reciprocal t(3;21) fusion transcripts in cases of acute leukemia (47). In addition, a recurrent pathogenic fusion oncogene arising from a t(7;17) rearrangement in endometrial soft tissue sarcoma was recently isolated in nontumor endometrial control samples with intact chromosomes 7 and 17 by cytogenetics and FISH resulting in the identification of physiologic *trans*-splicing of the same gene partners (43). An explanation for this observation proposed that the chimera may play a physiological growth-promoting role in normal development and the subsequent tumor specific t(7;17) rearrangement might then constitutively "lock-in" a tumor-promoting chimeric protein product resulting in neoplastic transformation. Similar findings were suggested, but not as convincingly shown, for BCR-ABL that have raised questions about the use of RT-PCR for minimal residual disease burden in patients with a clinical remission (49, 50). In addition, they also raise the question of whether *cis*- or *trans*-splicing transcripts may participate in the promotion of certain balanced chromosomal rearrangements or whether they both arise because of the same external effects, such as the relative spatial positioning of chromosomes (51). Although the detection of chimeric transcripts using DNAse-treated in vitro splicing extracts from different species suggests that chromosomal juxtaposition may not be an essential feature (43), further studies using other chimeric transcripts will need to be tested and validated. For example, another group recently reported the detection of EML4-ALK fusion transcripts by RT-PCR in 15% of normal lung tissues from patients with non-small

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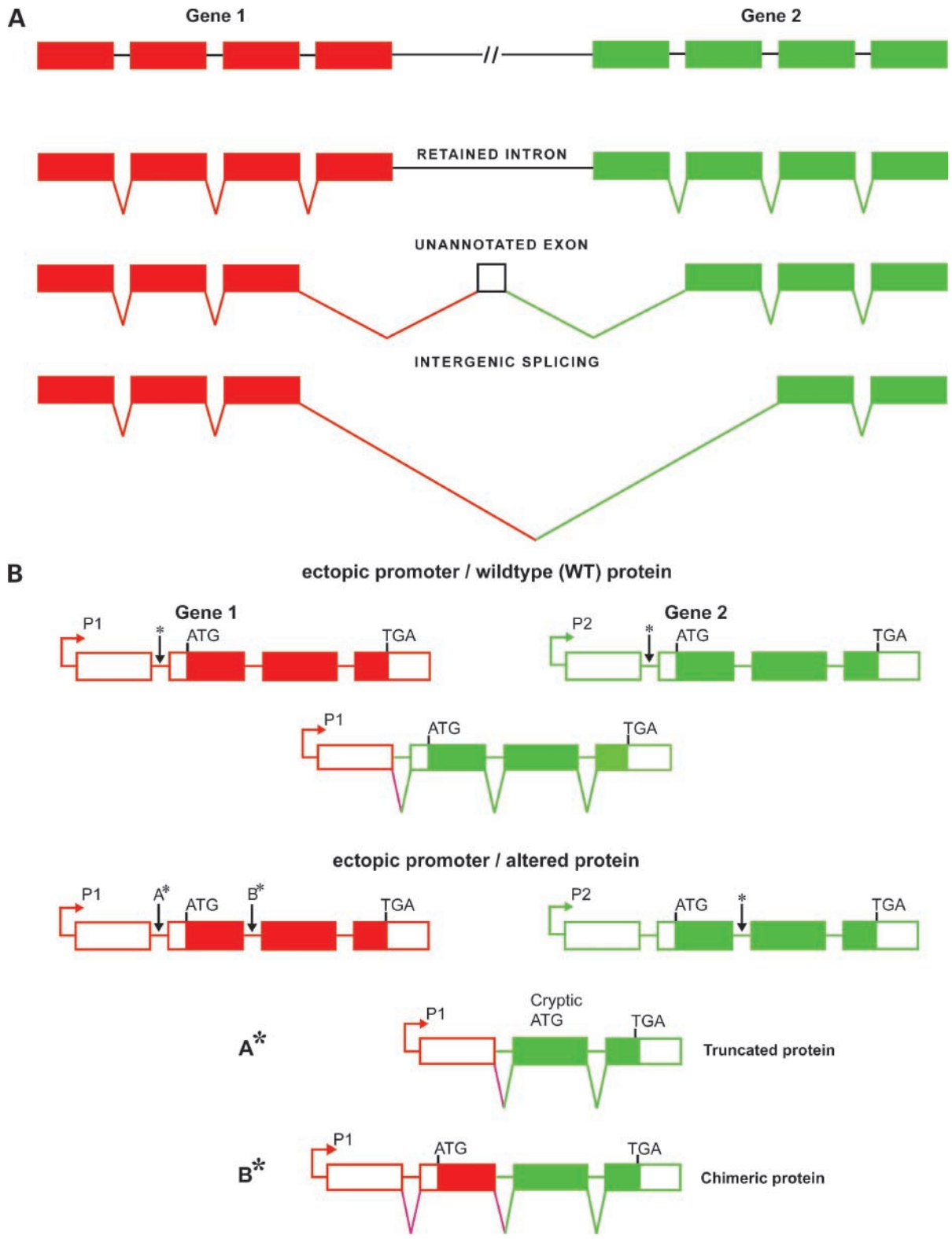

Figure 1. The chimeric transcriptome in health and disease. **A,** transcription-induced chimerism and intergenic or *cis*- and *trans*-splicing observed in both normal and tumor tissues. Cartoon depicts one splice isoform for each example, however transcription-induced chimerism may be associated with multiple variant isoform possibilities. **B,** mutation-associated fusion oncogenes. Cartoon depicts only one reciprocal fusion transcript. P1 indicates regulatory promoter element for Gene 1; arrows indicate locations of hypothetical chromosomal breakpoints; darker shades within exon boxes represent the encoded open reading frame.

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cell lung cancer (52). This study, however, showed ALK chromosomal rearrangements by break-apart FISH probes in scattered cells within histologically "normal" tissue rather than invoking *trans*-splicing. Therefore, confirming the extent and role of intergenic splicing in cell physiology and how it might relate to gene fusion oncogenesis will be important future goals.

Mutation-Associated Fusion Oncogenes

Promoter Swapping and Ectopic Expression of a Wild Type Gene Product

The simplest fusion event occurs with the group of chromosomal rearrangements in which the breakpoint is restricted to the 5′untranslated region resulting in ectopic overexpression of a wild-type, full-length downstream gene product. In the case of balanced translocations, this event is often referred to as "promoter swapping" and subsequent studies are required to identify which of the transcripts is the primary oncogenic "driver" mutation as the reciprocal fusion event may or may not be expressed. These studies may incorporate data from mRNA expression analysis, in vitro and in vivo tumorigenicity assays, targeted animal models, and inspection of patterns of homologous or related genes that might be involved in variant translocations within that histological subtype to ultimately identify the causal oncogene transcript(s). In contrast to hematological malignancies in which chromosomal breakpoints involving promoter or enhancer elements of the immunoglobulin and T-cell receptor genes are often located distant from their gene partner (53), promoter swapping of fusion oncogenes in solid tumor oncology generally includes a breakpoint that interrupts exons within the 5′untranslated region to generate a chimeric transcript that may be readily detected by RT-PCR strategies. Recent strategies to discover new fusion events in cancer often require the presence of these chimeric cDNA templates, which may result in an underrepresentation of rearrangements with distal promoter activation. In addition, there have been occasional examples of this event in solid tumors such as the juxtaposition of the PTH promoter more than 100 kb away from the cyclin D1 gene in a subset of parathyroid adenomas and carcinomas (54). Nonetheless, based on breakpoints data identified to date, most cases in solid tumors seem to involve some form of exon splicing between two gene partners. For example, salivary gland pleomorphic adenoma can arise through chromosomal translocations resulting in aberrant activation of the downstream wild-type PLAG1 gene product ectopically placed under the promoter and 5′ untranslated sequence of either the CTNNB1, LIFR, or TCEA1 genes (55). How PLAG1 mediates tumorigenicity (55) and why salivary gland tissue is targeted by these promoters is presently under investigation, however, tissue specificity for these regulatory elements is suggested by the observation that ectopic overexpression of wild-type PLAG1 by chromosomal rearrangements and promoter swapping with the alternate HAS2 or COL1A2 genes results in a different disease entity, lipoblastoma (56).

Promoter Swapping and Ectopic Expression of an Altered Gene Product

Promoter swapping or ectopic expression also readily lends itself for transcript detection by outlier gene expression screening, as discussed above, with the detection of ETV1 and ERG1 gene members targeted by chromosomal rearrangements in prostate adenocarcinoma samples (3). The breakpoints in these cases, however, also truncated the ETV1 and ERG1 *N*-terminal open reading frames serving the dual purpose of both altered gene expression and mutational activation of the protein product with a single mutational event. These types of combined alterations are the predominant form of chimeric oncogene seen in solid tumors and are likely to further enhance the histopathologic specificity of the fusion event. For example, it is predicted that TMPRSS2-ETV1 rearrangements will be detected exclusively in prostate cancer or other similar androgenic glandular tissues, although it remains to be seen if other minor variant partners of truncated ETV1, such as HNRPA2B 5′ regulatory elements, will show a restricted prostate tissue specificity. Accordingly, there is a growing sentiment to reclassify solid tumors on the basis of their pathogenic fusion translocations. For example, although the diagnosis of prostate cancer is not a major issue for pathologists, other tumor types such as undifferentiated midline carcinomas can raise difficult diagnostic and management issues and investigators have recently proposed renaming undifferentiated tumors carrying BRD3-NUT or BRD4-NUT fusion oncogenes as NUT midline carcinomas or NMC (57). This trend will continue with the recognition that the organization of common adult solid tumors by histology alone may be insufficient to capture the underlying genetic and biological heterogeneity in order to take full advantage of modern prognostic, predictive, and therapeutic tools.

Insights Learned from Mucoepidermoid Cancer

Although each of the fusion oncogenes isolated to date has unique genetic and biologic implications, there are several elements that are common among them. For example, the cloning of the Crtc1-Maml2 chimeric oncogene as the etiologic event for the most common subtype of malignant salivary gland tumor reinforced several important points about diagnosis, prognosis, and therapy that are generally applicable to the broad topic of fusion oncogenes in solid tumors.

Specificity Offers a Role for Diagnosis

Crtc1-Maml2 arises from a recurrent t(11;19) rearrangement in which 42 codons of the Crtc1 gene (exon 1) are fused in-frame with 981 codons of the Maml2 gene (terminal exons 2-5) to generate a chimeric peptide (6, 58). The cause of chromosomal translocations in solid tumors is generally unknown, however, mucoepidermoid cancer shows a strong radiation exposure dose-dependency (59–63) that resembles other radiation-associated tumors with chromosomal translocations (64), such as thyroid cancer, sarcomas, and certain

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chronic and acute leukemias. More than 150 primary mucoepidermoid cases have now been tested by RT-PCR and, although 50% to 70% of salivary gland mucoepidermoid tumors were fusion-positive overall, there was a marked difference between lower grade differentiated tumors (70%-90% fusion-positive) and undifferentiated tumors (<10% fusion-positive) (refs. 65–70). This observation suggested that many fusion-negative cases might represent a different histopathogenic entity, such as poorly differentiated adenosquamous carcinoma of the head and neck. In addition, because many RNA samples were extracted from archival paraffin embedded sections, it is predicted that the frequency of the fusion oncogene may exceed 90% if tested under optimal conditions in well differentiated tumors. Conversely, one of the fusion-positive tumors studied during the cloning of Crtc-Maml2 genes was initially interpreted as an adenosquamous lung tumor and only later reclassified as a pulmonary mucoepidermoid cancer (6). Therefore, especially for uncommon tumors in which pathology expertise may be limited, oncogene genotyping may allow more precise detection of cases and improve patient care.

Further evidence for a unifying concept of Crtc1-Maml2 expression was the subsequent observation that fusion-positive tumors were not restricted to major and minor salivary gland tissues, but have been detected within primary tumors with mucoepidermoid-like histology arising in the bronchopulmonary tree, the thyroid, breast, and cervix (6, 70–72). Tumors with a mucoepidermoid histology have also been occasionally detected in other organs throughout the body and it remains to be seen if they will also express Crtc1-Maml2 or variant homolog fusion partners. In addition, certain skin tumors with apocrine or eccrine (sweat gland) features that have been cataloged under numerous synonyms such as hidradenomas or eccrine or clear cell acrospiroma can also exhibit mucoepidermoid-like histologic features and have shown a high frequency of Crtc1-Maml2 positivity (73, 74). These findings suggest that Crtc1-Maml2, as well as Crtc3-Maml2 (75) and perhaps other variant homolog partners, may target mucous/serous glands scattered throughout the body for neoplastic transformation. An exception to this view is the observation that a distinct histologic type of salivary gland tumor, Warthins tumor, has been shown to also express Crtc1-Maml2 in a small subset of cases (65, 76). Because foci of mucoepidermoid cells have been known to arise de novo within Warthins tumor foci, it is still uncertain if these fusion-positive cases can occur in pure Warthins samples (66, 68). Another important exception was the detection of another fusion oncogene previously associated with undifferentiated sarcoma, EWSR1-POU5F1, which was detected in some cases of mucoepidermoid and hidradenoma (77).

Biology Offers a Role for Prognosis and Therapy

Fusion oncogenes arise in tumors as an early etiologic event that often confers an initial indolent clonal tumor expansion. For example, thyroid cancer and prostate cancer can present with an initial low-grade phase of tumor growth followed by more aggressive, poorly differentiated disease, and similar observations have also been noted with other translocation-associated diseases such as indolent B-cell lymphoma and the chronic phase of CML. In the case of RET-PTC thyroid cancer, mouse models have supported the prediction that the sequential accumulation of additional somatic mutations underlies the progression to higher grade cancer (78). Accordingly, Crtc1-Maml2 fusion positive mucoepidermoid tumors also show a generally indolent clinical behavior with prolonged survival following surgical resection of the tumor as compared with fusion-negative salivary gland tumors (67, 69). This information, therefore, may have important prognostic value to guide clinical decisions about surgical margins, which can affect cosmetic results, or the use of adjuvant radiation therapy. Fusion positive mucoepidermoid cancer, however, can progress to develop lethal stage 4 metastatic disease (701), and future studies will focus on generating mouse models and identifying the sequential somatic mutations that may underlie the transition to increased metastatic behavior. However, despite the eventual emergence of tumor cells with markedly increased aneuploidy, preclinical studies have shown that sustained expression of the chimeric oncogene is still required for tumor growth. For example, tumor cell lines derived from patients who died of stage 4 lung or parotid gland mucoepidermoid cancer showed >90% tumor colony growth inhibition when the Crtc1-Maml2 transcript was knocked-down by RNA interference (RNAi) methods (79). The same RNAi vectors had no growth effect on all other tumor cell lines that did not carry the t(11;19) rearrangement and single nucleotide changes in the RNAi sequence abrogated all tumor inhibition activity confirming that the chimeric transcript is a bona fide target for therapeutic strategies. Although localized mucoepidermoid cancer might serve as an excellent model for developing efficient in vivo RNAi delivery methods in a regionally localized head and neck tumor, there are still numerous obstacles for this approach. Therefore, defining the biology of the fusion chimera and its corresponding gene partners may identify downstream candidate targets that are suitable for more traditional drug-able therapies. Initial functional studies focused on the Maml2 domain, an essential co-activator for Notch receptor signaling (6), however global expression microarray profiling following Crtc1-Maml2 induction in mammalian cells showed activation of cAMP/CREB inducible genes and not Notch target genes (80, 81). The independent isolation of Crtc gene members as a CREB co-activator and potent mediator of anabolic metabolism (82) and the observation that mini-deletions within the small Crtc1 domain that blocked CREB binding also abrogated transformation potential of Crtc1-Maml2 in RK3E cells (80) has refocused attention on Crtc1 as a potential link between energy metabolism and cancer. Ultimately, these data also suggest that the development of strategies to inhibit Crtc transcriptional activity in mucoepidermoid cancers may offer potential benefit to other non-salivary gland malignancies in which this new signaling

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pathway may be aberrantly activated by alternate, nontranslocation mechanisms.

Conclusions

There has been a rapid increase in the rate of identification of novel fusion oncogene transcripts in solid tumors and the widespread application of noncytogenetic based methods predicts more discoveries over the next years. These global nonbiased assays will also serve to annotate and advance our understanding of the functional role for an unexpectedly large number of chimeric transcripts that are being detected in normal tissues and that may force us to reassess: (1) our long-held views on how to define a gene and (2) if fusion oncogenes may represent constitutive forms of a physiological chimeric transcript (43), which may explain why so many translocation-associated tumors present with an initial indolent growth phase. Although new data on fusion oncogenes in solid tumors can be readily incorporated into revised diagnostic classifications, the ultimate challenge will be to develop effective therapeutic strategies for patients on the basis of this knowledge.

Disclosure of Potential Conflicts of Interest

F. Kaye is a coinventor on a patent for molecular diagnosis of Crtc1-Mam12 tumors.

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Molecular Cancer Therapeutics

Mutation-associated fusion cancer genes in solid tumors

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