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FISH molecular testing in cytological preparations from solid tumors

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Abstract

Many of the exciting new developments in solid tumor molecular cytogenetics impact classical and molecular pathology. Fluorescence in situ hybridization to identify specific DNA target sequences in nuclei of non-dividing cells in solid neoplasms has contributed to the integration of molecular cytogenetics into cytology in spite of the remarkable promiscuity of cancer genes. Indeed, although it is a low-throughput assay, fluorescence in situ hybridization enables the direct disclosure and localization of genetic markers in single nuclei. Gene fusions are among the most prominent genetic alterations in cancer, providing markers that may be determinant in needle biopsies that are negative or suspicious for malignancy, and may contribute to the correct classification of the tumors. In view of the expanding use of fluorescence in situ hybridization in cytology, future challenges include automated sample evaluation and the specification of common criteria for interpreting and reporting results.

Keywords: FISH, Gene fusions, Cytology, Solid tumor, Gene promiscuity

Background

Various types of genetic alterations, as well as epigenetic phenomena, have been identified and are now considered important in the classification, prognosis, and treatment of cancer. Correlations between genomic instability and carcinogenesis have been extensively investigated, leading to the recognition of an increasing number of genetic abnormalities as a tumor driving force. Currently, several molecular approaches are available to investigate tumor cell pathobiology at different levels (chromosome, gene, gene expression). The predominant approaches include immunohistochemistry, fluorescence *in situ* hybridization (FISH), polymerase chain reaction, array-based and omics-based techniques [1-5]. The integration of results obtained using these platforms has been invaluable in clarifying genetic alterations associated with cancer and in interpreting the key role of the impaired signaling pathways. Gene gains and losses and gene disruptions by chromosome translocation, inversion, or deletion have been recognized as playing a pathogenetic role in many cancers. These exciting new developments in solid tumor molecular cytogenetics impact classical and molecular pathology, and an increasing number of chromosome markers have been integrated into World Health Organization tumor classifications [6]. Some of these markers are also relevant to selection of therapies targeting the protein products of gene fusions. In this scenario the impact of testing gene alterations by interphase FISH in material from needle biopsies and organic fluids has rapidly increased.

Promiscuity: a false dilemma?

The identification of a specific translocation in solid tumors dates back to 1983 when the t(11;22)(q24;q12) in Ewing's sarcoma was first described [7]. It took nine years before the underlying gene fusion, *EWS/FLI1* [8] (today named *EWSR1/FLI1*), was discovered. The *EWS/FLI1* fusion was found to be closely associated with this type of sarcoma, and was thought to play a causal role in initiating the neoplastic process. Subsequent observations of variant translocations and the resulting *EWSR1* fusions with different partner genes in the same tumor entity disclosed the tip of an iceberg, paving the way for discovering the phenomenon of gene promiscuity in cancer. Indeed, the molecular cytogenetics of Ewing's sarcoma family tumors (so called ESFT) and subsequently of other histologically unrelated soft tissue tumors, and finally of tumors arising in tissues of distinct embryological origin, demonstrated the ubiquitous involvement of the

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PARTNER of *EWSR1*	TUMOR
ZNF384	Acute leukemia
ATF1, CREB1	Angoimatoid fibrous histiocytoma
ATF1, CREB1	Clear cell sa.
ATF1, CREB1	Clear cell sarcoma-like tu. GI tract
WT1	Desmoplastic small round cell tu.
NR4A	Extrascheletal myxoid condrosarcoma
EIAF, ERG, ETV1, FLI1, FEV, NFATC2, PATZ1, SMARCA5, SP, ZNF278	Ewing sa.
ATF1	Hyalinising clear cell ca. salivary gland
CREB3L1	Low grade fibromyxoid sa.
ATF1	Malignant melanoma
YY1	Mesothelioma
POU5F1	Mucoepidermoid ca.a salivary gland
PBX1, POU5F1, ZNF44, ATF1	Myoepithelioma soft tissue
DDIT3	Myxoid liposarcoma
ATF1,CREB1	Primary pulmonary mixoid sa.
POU5F1	Skin hydroadenoma
CREB3L1	Sclerosing epitheliod fibrosarcoma

Epithelial lineage; Hematological lineage; Mesenchymal lineage; Mesenchymal and Epithelial lineage; ca. = carcinoma; sa. = sarcoma; tu. = tumor

Figure 1 Promiscuity of the *EWSR1* gene in malignancy.

PARTNER of *ALK*	TUMOR
ALO17, ATC, MYH9. MSN, NPM1, RNF213, TFG, TPM3	Anaplastic large cell lymphoma
ELM4	Breast cancer
EML4	Colon cancer
A2M	Fetal lung interstitial tu.
ATC, CARS, CLT1, TPM3, TPM4, RANPB2, SEC31A	Inflammatory myofibroblastic tu.
VCL	Medullary kidney ca.
RANBP2	Myeloproliferative disorders
ELM4, C2orf44, HIP1, K1F5B, TGF, TRP, ROS1	Non-small cell lung ca.
TPM4	Oesophageal squamous cell ca.
FN1	Ovarian stromal sa.
TPM3	Systemic Histiocytosis
CLTC, NPM1, SEC31A, SQSTM1	Subgroup DLBCL
EML4, STRN	Thyroid ca.
PARTNER of *BCOR*	**TUMOR**
RARA	Acute leukemia
ZC3H7B	Endometrial stromal sa.
CCNB3	Sarcoma Ewing-like
PARTNER of *ETV6*	**TUMOR**
ABL1, ACSL1, ACSL6, ARNT, CHIC2, JACK2, MN1, NCOA2, NTRK3, PAX5, PDGFRA, PDGFRB, R*UNX1, TTL*	Acute leukemia
NTRK3	Congenital mesoblastic lymphoma
NTRK3	Infantile fibrosarcoma
NTRK3	Secretory breast ca.
NTRK3	Secretory ca. of salivary gland
NTRK3	Thyroid ca.
PARTNER of *FGFR* (*FGFR-Member*)	**TUMOR**
TACC3, BAIAP2L1(FGFR3)	Bladder ca.
ERLIN2 (FGFR1); AFF3, CASP7, CCDC6 (FGFR2)	Breast ca.
CEP110 (FGFR1)	Mieoproliferative syndrome
BAG4 (FGFR1); BICC1, TACC3, CIT, KIAA1967(FGFR2)	Non-small cell lung cancer
TACC1(FGFR1); TACC3(FGFR3)	Glioblastoma
BICC1(FGFR2)	Metastatic colangiocarcinoma
SLC45A3 (FGFR2)	Prostate ca.
OFD1(FGFR2)	Thyroid ca.

Epithelial lineage; Hematological lineage; Mesenchymal lineage; Neuroepithelial lineage; ca. = carcinoma; sa.= sarcoma; tu. = tumor

Figure 2 Promiscuity of the *ALK, BCOR, ETV6, FGFR* genes in malignancy.

EWSR1 gene in a wide spectrum of cancers, from sarcoma to carcinoma and to hematological malignancy (Figure 1). It is now clear that most structural gene alterations mediated by chromosome rearrangements (examples in Figures 2 and 3) [9,10], drive malignancy in a variety of tumors of different hystogenetic types. Nevertheless, this evidence does not invalidate the role of gene fusions or deregulated genes as diagnostic tools. Indeed, the accumulated data prompted expansion of the understanding of gene promiscuity. For example, it is now clear that the *EWSR1* gene fuses with several genes mainly encoding transcriptional regulator factor families, resulting in deregulation of specific molecular pathways. These include ETS, homeobox-genes, zinc finger, and leucine-zipper transcription factor families. Disruption of these pathways may influence the pathogenesis of specific tumor types through a variety of activation mechanisms [11]. In spite of promiscuity, searching for gene involved in chromosome alterations leading to illicit shuffling of coding or regulatory sequences in cancer is becoming an invaluable approach in cytological investigations as well.

Cytology and cytogenetics

Based on the above scenario, the relationship between cytology and cytogenetics has become increasingly close, and the use of cytogenetics as an ancillary supplementary tool in cytological diagnosis has been introduced in the pathology sector. In particular, the leitmotif of the union of cytology and cytogenetics [12] is the need for a close collaboration between the two parts, since on one hand asking for a FISH test implies being aware of the rearrangement to be investigated (Figure 4), and on the other the cytogenetic result needs to be interpreted in the context of the cytological (and possibly clinical) observations. In addition, a FISH test is often used as a confirmatory tool since a negative result is not informative, both because unknown alterations cannot be excluded and the availability of tumor cells in cytological preparations may be limited.

Considering the introduction of systematic genomic testing for some tumors (such as lung and breast cancer) [13,14], the consequent need for a correct evaluation of ratio value in the presence of genetic heterogeneity [15], and the growing demand for FISH tests in fine needle aspirations and organic fluids, two main challenges for the future can be foreseen: the implementation of automated FISH evaluation and the specification of common criteria for interpreting and reporting FISH results in as many tumor types as possible. A significant impediment to evaluating the ever increasing numbers of clinical FISH tests requested is imposed by the labor intensive nature of the assay, as each test requires scoring numerous interphase nuclei by double blind observation. Automated FISH, with strictly established parameters for standardization, could partly overcome these issues, although automation has yet to be perfected [16]. Specific recommendations and guidelines for FISH on tumors have been established within ACMG (American College of Medical Genetics and Genomics) [17] and E.C.A (European Cytogeneticists Association) [18]. On

PARTNER of *NCOA2*	**TUMOR**
ETV6, MYSTR3	Acute leukemia
PAX3	Alveolar rabdomyosarcoma
AHRR, GTF21	Angiofibroma
HEY1	Chondrosarcoma
PARTNER of *PHF1*	**TUMOR**
RARA	Acute promyelocytic leukemia
CCNB3	Ewing sarcoma-like
ZC3H7B	Endometrial stromal sa.
PARTNER of *RET*	**TUMOR**
BCR, FGFR1OP	Chronic myelomonocytic leukemia
CCDC6, KIF5B, NCOA, CUX1	Non-small cell lung cancer
CCD6, ELKS, Golgas, HOOK3, NCOA4, KTN1, RFG9, PCM1, PRKAR1A, TRIM24, TRIM27, TRIM33	Thyroid papillary ca.
PARTNER of *TFE3*	**TUMOR**
ASPSCR1	Alveolar soft part sa.
YAP1	Epithelioid haemangioepithelioma
RREB1	Inflammatory myofibroblastic tu.
PSF	Perivascular epithelioid cell tu.
ASPSCR1, PRCC, PSF, NonO, CLTC	Xp11-renal cell ca.

Epithelial lineage; Hematological lineage; Mesenchymal lineage; ca. = carcinoma; sa.= sarcoma; tu. = tumor

Figure 3 Promiscuity of the *NCOA2, PHF1, RET, TFE3* genes in malignancy.

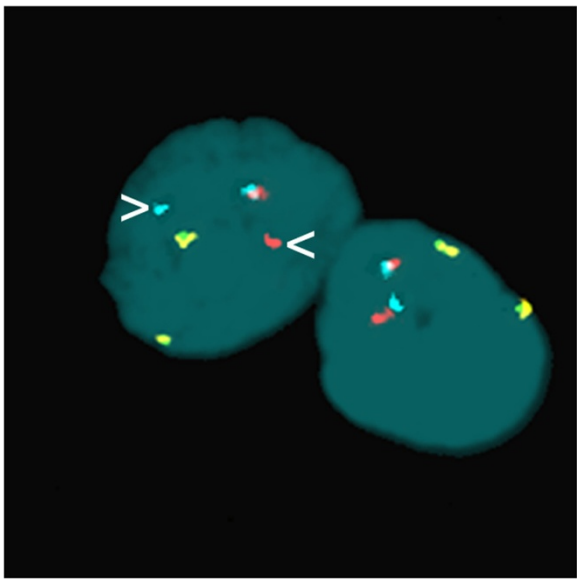

Figure 4 Example of FISH in a cytological preparation. A cytological preparation from thyroid fine needle aspiration was simultaneously hybridized with *RET* (labeled with Spectrum Aqua/Spectrum Red) and *PPARg* (labeled with Spectrum Green/Spectrum Gold respectively) split-apart probes. Broken *RET* is revealed by the split-apart centromeric aqua and telomeric red (arrowheads) probes. Contiguous dual-color signals indicate intact genes. Nuclei are counterstained with DAPI.

the other hand, common objective interpretation criteria for FISH on cytological preparations, as well as quality control and quality assurance policies, remain limited [13,14], and require an extraordinary cooperative effort and interaction between cytogeneticists and cytologists. It would be desirable to convene expert advisory panels from scientific societies of clinical cytogeneticists and pathologists to establish evaluation criteria for the various tumors, based on expertise and a review of published literature, with a view to establishing common shared recommendations.

Conclusions

Many of the exciting new developments of molecular cytogenetics are having a profound impact on classical and molecular cytology. The growing demand for cytological FISH tests highlights the need for the specification of common criteria for interpreting and reporting FISH results, for quality control and quality assurance policies, and for possible implementation of automated FISH evaluation.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

PC and RV participated in commentary design and wrote the manuscript. They read and approved the final manuscript.

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