Fluorescence in situ hybridization in surgical pathology: principles and applications

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Abstract

Identification of recurrent tumour-specific chromosomal translocations and novel fusion oncogenes has important diagnostic, therapeutic and prognostic implications. Over the past decade, fluorescence in situ hybridization (FISH) analysis of tumour samples has been one of the most rapidly growing areas in genomic medicine and surgical pathology practice. Unlike traditional cytogenetics, FISH affords a rapid analysis of formalin-fixed, paraffin-embedded cells within a routine pathology practice workflow. As more diagnostic and treatment decisions are based on results of FISH, demand for the technology will become more widespread. Common FISH-detected alterations are chromosome deletions, gains, translocations, amplifications and polysomy. These chromosome alterations may have diagnostic and therapeutic implications for many tumour types. Integrating genomic testing into cancer treatment decisions poses many technical challenges, but rapid progress is being made to overcome these challenges in precision medicine. FISH assessment of chromosomal changes relevant to differential diagnosis and cancer treatment decisions has become an important tool for the surgical pathologist. The aim of this review is to provide a theoretical and practical survey of FISH detected translocations with a focus on strategies for clinical application in surgical pathology practice.

Keywords: fluorescence in situ hybridization; molecular genetics/cytogenetics; targeted therapy; precision medicine; differential diagnosis

Introduction

In the late 1960s, in situ hybridization was first performed with radioisotope-labelled probes followed by autoradiography [1–3]. Fluorescence-labelled probe technology started in the early 1980s with RNA probes that were directly labelled with fluorophores complementary to specific DNA sequences. Advances in fluorescence microscopy and digital imaging, as well as widespread availability of genomic and bioinformatic resources, have greatly improved the resolution, sensitivity, specificity, and accessibility of fluorescence in situ hybridization (FISH).

Interphase FISH, hereinafter simply referred to as FISH, is the usual clinical application of this diagnostic tool [4]. FISH involves the use of fluorescence labelled fragments of DNA (probes) binding to interphase chromosomes of cytology specimens or paraffin embedded tissue sections. Common FISH-detected alterations are chromosome deletions, gains, translocations, amplifications and polysomy. These chromosome alterations may have diagnostic and therapeutic implications for many tumour types [5,6]. Integrating precise genomic testing into cancer treatment decisions poses technical challenges, but rapid progress is being made in overcoming these difficulties. This review focuses on FISH based detection of translocations applicable to surgical pathology.

Chromosomal translocations: an overview

Chromosomal translocation refers to a chromosome rearrangement involving non-homologous chromosome
pairs. Translocations can be balanced, without net gain or loss of material, or unbalanced, with gain or loss of genetic loci. Translocation often creates fusion genes when two otherwise separated chromosome parts join [7–9]. These fusion genes may behave as a hybrid chimeric oncogene, inducing tumorigenesis by gene overexpression, or they may interrupt an important control region, causing unresponsiveness to regulatory control factors. For example, receptor tyrosine kinase gain of function fusion oncogene is a mechanism whereby mesenchymal cells may be transformed into sarcomas. Constitutive activation of the insulin family kinase ALK results from unregulated dimerization of the fusion gene protein containing the active anaplastic lymphoma kinase (ALK) kinase domain. This mechanism underlies about half of inflammatory myofibroblastic tumours. Most Ewing sarcoma cases result from EWSR1-FLI1 gene fusion deregulating the ETS transcription factor FLI1, leading to aberrant expression of Ewing sarcoma transforming genes. The pathognomonic fusion protein of dermatofibrosarcoma protuberans (DFSP) is PDGFB-COL1A1. This amalgamation of the essential fibroblast collagen gene (COL1A1) with the angiogenic growth factor gene (PDGFB) leads to increased growth factor release. An overabundance of growth factor results in overstimulation of receptor tyrosine kinase PDGFR, activating not only endothelial cells but also fibroblasts and inflammatory cells [10].

Translocations may involve two or more different chromosomes or may exchange information between regions in the same chromosome. Intra-chromosomal rearrangement may cause transposition of the involved loci with or without centromere involvement. Chromosome rearrangement, whether intra-chromosomal or between chromosomes, invariably leads to a neoteric juxtaposition of previously unrelated gene elements into a new fusion gene that may or may not be transcribed. Fusion gene detection, therefore, is at the heart of FISH diagnostic testing. Chromosomal translocation is detectable by several methods, including FISH, metaphase karyotyping, microarray comparative genomic hybridization, SNP-array and next generation sequencing [10–20]. Most chromosomal translocations are detectable by FISH, either by split-apart (Figure 1) or fusion probes (Figure 2). To be a reliable diagnostic tool, the probe sets must reproducibly show either an increase or a decrease in the optical distance between probe signals. Some translocations, especially those involving intra-chromosomal inversion or deletion, have a relatively short physical distance between the fusion partners after translocation. These small distances cannot reliably be resolved at the light microscopic level. In this scenario, a PCR-based method or RNA sequencing (RNA-Seq) is favoured. RNA-seq, a next generation sequencing technique, can detect both known and novel gene fusion events and allows RNA analysis through cDNA sequencing at a comprehensive genome-wide scale. It can detect both cryptic intrachromosomal rearrangements and fusion products with uncharacterized fusion partners [14,21–26].

Chromosomal translocations mainly associated with sarcomas

About 30% of sarcomas have well documented specific translocations, and the percentage continues to grow. With some exceptions, sarcoma translocations form fusion genes with the 5’ gene segment contributing a strong promoter and overexpression of the 3’ proto-oncogene segment by transactivation. Recurrent gene fusions in soft tissue sarcomas tend to occur in tumours sharing similar morphological characteristics [27]. Roughly half of the sarcoma-associated fusion genes contain a member of the TET gene family, including FUS, Ewing sarcoma breakpoint region 1 (EWS1) is the promoter and TAF15 is the proto-oncogene partner. Most fusion genes are strongly associated with a particular tumour type, potentially making them ideal molecular diagnostic markers [10,27–29]. Some fusion proteins are actual or potential therapeutic targets, making the detection of fusion gene products valuable both for diagnostic and for therapeutic purposes.

More than 400 recurring translocations have been found in soft tissue sarcomas. The challenge now is to identify which of these recurring translocations truly have diagnostic, mechanistic, or therapeutic meaning for the associated sarcomas. Over 30 subtypes of mesenchymal tumour can be confirmed by FISH analysis based on tumour-specific chimeric fusion sequences [28]. FISH-identifiable sarcomas include many common entities: Ewing sarcoma/primitive neuroectodermal tumour, synovial sarcoma, liposarcoma, round cell Ewing-like sarcoma, pulmonary myxoid sarcoma, inflammatory myofibroblastic tumour and others (Table 1).

Ewing sarcoma

Ewing sarcomas are composed of small round blue cells with highly malignant behaviour, usually affecting bone and extraosseous tissues of children and young adults. There is a specific balanced chromosomal rearrangement in most Ewing sarcomas,
providing a valuable tool for diagnosis. The fusion partners of EWSR1 belong to several gene families, mainly encoding transcription factors. These proteins join the amino terminal of the EWSR1 gene with the carboxy terminal of an ETS gene family member. This fusion not only abrogates the RNA splicing function of EWSR1 but also removes essential trans-activating control regions of the ETS gene partner. As many as 90% of the translocations involve the EWSR1 gene in the form of t(11;22)(q24;q12) translocation, which juxtaposes EWSR1 at 22q12 with the FLI1 gene on chromosome 11q24 [30].

The fusion gene encodes a protein containing the upstream amino-terminal domain of EWSR1 and the downstream carboxy-terminal region of FLI1. This fusion protein uniquely functions in controlling cell growth and regulating the transcription of downstream genes responsible for uncontrolled cell division, and survival in the face of otherwise lethal additional mutations. The ERG translocation, t(21;22)(q22;12), resulting in EWSR1-ERG fusion, represents only 10% of all Ewing sarcomas. The functional consequences of these variant fusion proteins are similar to those of the more common EWSR1-FLI1 fusion. One study suggested a better outcome for patients with localized tumours expressing the most common t(11;22) transcript (EWSR1 exon 7 fused to FLI1 exon 6), but limited prognostic

Figure 1. Break-apart probe design for translocations with multiple fusion partners. A break-apart probe set is composed of two probes specific for loci physically close to each other on the chromosome in their wild type configuration. The wild type signal pattern shows two pairs of closely approximated or fused signals (A). When translocation occurs involving a breakpoint between the two probe sites, the originally juxtaposed loci (fusion signals) split apart (B). The beauty of this design is that it detects chromosomal translocation regardless of the fusion partner involved. However, the break-apart probe only identifies the breaking away of a gene fragment from its original location. It does not determine which chromosome receives the fragment or which genes may serve as fusion partners in the new location.
data is currently available for the less common fusion types [31].

Myxoid liposarcoma

The diagnosis of myxoid liposarcoma may be challenging by histology alone, especially on small biopsy tissues, since a variety of soft tissue tumours with myxoid change may mimic myxoid liposarcoma. Myxoid liposarcoma represents about 10% of all adult sarcomas and approximately 33% of all liposarcomas. Chromosomal translocations t(12;16)(q13;p11) and t(12;22)(q13;q12), rendering gene fusions of DNA-damage inducible transcript 3 (DDIT3) with FUS or EWSR1, respectively, are characteristic of myxoid liposarcoma; one of these

Figure 2. Fusion design for translocation between specific partners. A fusion probe set contains two locus-specific probes labelled with different colours targeting the genes known to fuse in certain tumours or diseases. The fusion probes identify chromosomal loci far from each other either on the same chromosome or on different chromosomes. The wild type configuration shows two separated signals resulting from physically separated chromosome loci (A). When fusion occurs, the two probes move to a closely approximated position (fusion signal, B). This provides direct evidence of a gene fusion with definitive fusion partners identified.
| Tumour Type                                      | Chromosomal Translocation | Genes Involved                | Frequency (%) |
|-------------------------------------------------|---------------------------|--------------------------------|---------------|
| **Adipocytic tumours**                          |                           |                                |               |
| Chondroid lipoma                                | t(11;16)(q13;p13)         | C11orf95-MKL2                  | 75-85         |
| Lipoma                                          | t(3;12)(q27;q14)          | HMGA2-LPP                      | 20-25         |
|                                                | t(12;13)(q13;q14)         | HMGA2-LHFP*                    |               |
|                                                | t(3;13)(q27;q14)          | LPP-LHFP*                      |               |
|                                                | t(12;12)(q12;q14)         | 7-HMG-A2*                      |               |
| Lipoblastoma                                    | t(2;8)(q31;q12)           | COL3A1-PLAG1                   | 50-75         |
|                                                | t(8;3)(q24;q12)           | HAS2-PLAG1*                    |               |
|                                                | t(7;8)(q11;q12)           | COL1A2-PLAG1                   |               |
|                                                | t(8;14)(q12;q24)          | RADS1B-PLAG1                   |               |
| Spindle cell/pleomorphic lipoma                 | - 13/del(13)(q14)         | RB1                            | 40-48         |
|                                                | del(16)(q13)              | -                              | 40-50         |
| Hibernoma                                       | del(11)(q13-21)           | MEN1                           | 66-80         |
| Atypical lipomatous tumour/well differentiated   | amp(12)(q14-15)           | MDM2                           | 80-90         |
| liposarcoma                                     | Supernumerary ring        | -                              | 75-90         |
| Myxoid liposarcoma                              | t(12;16)(q13;p11)         | FUS-DDIT3                      | 90-95         |
|                                                | del(10)(q23)              | PTEN                           | 7-12          |
|                                                | t(12;12)(q13;q12)         | EWSR1-DDIT3*                   |               |
|                                                | t(12;12;20)(q13;q12;q11)  | EWSR1-DDIT3                    |               |
| **Fibroblastic/myofibroblastic tumours**        |                           |                                |               |
| Nodular fasciitis                               | t(17;22)(p13;q13)         | MYH9-USP6                      | 90-92         |
| Elastofibroma                                   | amp(X)(q12-q22)           | -                              | 16-30         |
| Fibroma of tendon sheath                        | t(2;11)(q31;q12)          | -                              | #             |
|                                                | t(9;11)(p24;q13)          | -                              | #             |
| Desmoplastic fibroblastoma                      | t(2;11)(q31;q12)          | -                              | #             |
|                                                | t(11;17)(q12;p11)         | -                              | #             |
| Cellular angiofibroma                           | del(13)(q14)              | RB1, FOXO1                     | 54-80         |
| Mammary-type myofibroblastoma                   | del(13)(q14)              | RB1, FOXO1                     | 70-92         |
| Tenosynovial giant cell tumour                  | t(12;12)(p13;q37)         | CSF1-COL6A3                    | 50-80         |
| Giant cell fibroblastoma                        | t(17;22)(q22;q13)         | COL1A1-PDGFB                   | 80-92         |
| Dermatofibrosarcoma protuberans                 | t(17;22)(q22;q13)         | COL1A1-PDGFB                   | 74-90         |
|                                                | Supernumerary ring        | -                              | 50-65         |
| Inflammatory myofibroblastic tumour             | t(1;2)(q21;p23)           | TPM3-ALK                       | 25-30         |
|                                                | t(2;19)(p23;p13)          | TPM4-ALK                       |               |
|                                                | t(2;17)(p23;q23)          | CLTC-ALK                       |               |
|                                                | t(2;11)(p23;p15)          | CAR5-ALK                       |               |
|                                                | inv(2)(p23;q35)           | ATIC-ALK                       |               |
|                                                | t(2;4)(p23;q27)           | SEC31A-ALK                     | 10-20         |
|                                                | t(2;12)(p23;p12)          | PPIFIB1-ALK                    |               |
|                                                | t(2;5)(p23;q35)           | NPM1-ALK                       |               |
|                                                | t(2;2)(p21;p23)           | EML4-ALK                       |               |
|                                                | t(2;1)(p21;q21)           | TPM3-ALK                       |               |
|                                                | t(2;19)(p21;p13)          | TPM4-ALK                       |               |
|                                                | t(2;22)(p23;q13)          | RANBP2-ALK                     |               |
|                                                | t(6;3)(q22;q12)           | TFG-ROS1                       | 6-10          |
|                                                | t(6;17)(q22;p13)          | YWHAE-ROS1                     |               |
|                                                | t(5;12)(q32;q13)          | NAB2-PDGFRB                    |               |
| Low grade fibromyxoid sarcoma                   | t(7;16)(q33;p11)          | FUS-CREB3L2                    | 60-80         |
|                                                | t(11;16)(p11;p11)         | FUS-CREB3L1                    | 3-6           |
|                                                | Supernumerary ring        | -                              | 25-42         |
| Tumour Type | Chromosomal Translocation | Genes Involved | Frequency (%) |
|-------------|--------------------------|----------------|--------------|
| **Myoinflammatory fibroblastic sarcoma** | t(1;10)(p22;q24) | TGFBR3-MGEA5 | 62-80 |
| | amp(3)[p12] | VGLL3 | 37-50 |
| **Infantile fibrosarcoma** | t(12;16) (p13;q25) | ETV6-NTRK3 | 75-90 |
| **Solitary fibrous tumour** | inv(12)(q13q13) | NAB2-STAT6 | 44-55 |
| **Congenital fibrosarcoma** | t(12;15)(p13;q25) | ETV6-NTRK3 | 90-99 |
| **Sclerosing epithelioid fibrosarcoma** | t(11;22)(p11;q12) | EWSR1-CREB3L1 | 80-90 |
| | t(12;16)(q13;p11) | DDIT3-FUS | 10-20 |
| | t(7;16)(q33;p11) | FUS-CREB3L2 | * |
| | t(11;16)(p13;p11) | FUS-CREB3L1 | * |
| **So-called Fibrohistiocytic tumours** | | | |
| **Tenosynovial giant cell tumour** | t(1;2)(p11;q35-36) | CSF1-COL6A3 | 50-64 |
| **Epithelioid fibrous histiocytoma** | t(2;10)(q22;p22) | VCL-ALK | 80-88 |
| | t(2;5)(p23;q35) | SQSTM1-ALK | * |
| **Smooth muscle tumours** | | | |
| **Benign metastasizing leiomyoma** | del(19)(q13.3), del(22)(q12) | – | # |
| **Uterine leiomyoma** | t(12;14)(q14;q22) | HMGA2-ALDH2 | 17-20 |
| **Pericytic (perivascular) tumour** | t(7;12)(p22;q13) | ACTB-GLI1 | # |
| **Embryonal rhabdomyosarcoma** | del(11p15.5) | IGF2, H19, CDKN1C, HOTS | 35-45 |
| **Alveolar rhabdomyosarcoma** | t(2;13)(q35;q14) | PAX3-FOXO1 | 60-85 |
| | t(1;13)(p36;q14) | PAX7-FOXO1 | 15-20 |
| | t(2;2)(q35;p23) | PAX3-NCOA1 | 10-18 |
| | t(2;8)(q35;q13) | PAX3-NCOA2 | * |
| | amp(12)(q13-15) | GLI1, CDK4, MDM2 | 22-56 |
| | amp(2)(p24) | MYCN | 16-50 |
| **Vascular tumours** | | | |
| **Epithelioid haemangioma** | t(1;3)(p36;q25) | WWTR1-CAMTA1 | 70-90 |
| | t(8;11)(p11;q22) | YAP1-TEF3 | * |
| | t(19;19)(q13;q13) | ZFP36-FOSB | * |
| | t(19;11)(q13;q22) | FOS-LMNA | * |
| | t(19;10)(q13;p13) | FOS-VIM | * |
| **Pseudomyogenic haemangiendothelioma** | t(7;19)(q22;q13) | SERPINE1-FOSB | 20-30 |
| **Epithelioid hemangiendothelioma** | t(11;X)(q22;p11) | YAP1-TEF3 | 80-94 |
| | t(1;3)(p36;q23) | WWTR1-CAMTA1 | 77-85 |
| **Chondro-osseous tumours** | | | |
| **Chondromyxoid fibroma** | inv(6)(p25q13) | – | 75-80 |
| | del(6)(q24) | – | 20-40 |
| | t(6;17)(q23;p13) | BCLAF1-RAP1GAP2 | 15-20 |
| | t(6;9)(q25;p22) | COL12A1-? | 6-20 |
| **Mesenchymal chondrosarcoma** | del(8)(q21)/t(8;8)(q21;q13) | HEY1-NCOA2 | 70-80 |
| **Nerve sheath tumours** | | | |
| **Melanotic schwannoma** | del(2)(p16)/amp(2)(p16) | CNC2 | 70-80 |
| **Perineurioma** | - del(22)(q12) | NF2 | 75-80 |
| | Supernumerary ring | – | |

Table 1. Continued

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| Tumour Type | Chromosomal Translocation | Genes Involved | Frequency (%) |
|-------------|--------------------------|----------------|--------------|
| Schwannoma  | 22q                      | NF2, SMARCB1   | 40           |
| Ewing sarcoma | t(11;22)(q24;q12)       | EWSR1-FLI1    | 80-90        |
|             | t(21;22)(q22;q12)       | EWSR1-ERG     | 12-15        |
|             | t(7;22)(p2;q12)         | EWSR1-ETV1    | 5-10         |
|             | t(17;22)(q21;q12)       | WESR1-ETV4    | *            |
|             | t(2;22)(q35;q12)        | EWSR1-FEV     | *            |
|             | t(20;22)(q13;q12)       | EWSR1-NFATC2  | *            |
|             | inv(22)(q12;q12)        | EWSR1-ZFG     | *            |
|             | t(4;22)(q31;q12)        | EWSR1-SMARCA5 | *            |
|             | t(6;22)(p21;q12)        | EWSR1-POUF1   | *            |
|             | t(2;22)(q31;q12)        | EWSR1-SP3     | *            |
|             | t(12;22)(q13;q21)       | EWSR1-PATZ    | *            |
|             | t(16;21)(p11;q22)       | FUS-ERG       | *            |
|             | t(2;16)(q35;p11)        | FUS-FEV       | *            |
|             | del(9)(p21)             | CDKN2A        | *            |
|             | t(4;19)(q35;q13)        | CIC-DUX       | *            |
|             | t(X;X)(p11;p11)         | BCOR-CCNB3    | *            |
| Tumours of uncertain differentiation | | | |
| Angiomatoid fibrous histiocytoma | t(2;22)(q33;q12) | EWSR1-CREB1 | 75-85 |
|             | t(12;22)(q13;q12)       | EWSR1-ATF1    | 10-14        |
|             | t(12;16)(q13;p11)       | FUS-ATF1      | *            |
| Exraskeletal myxoid chondrosarcoma | t(9;22)(q22;q12) | EWSR1-NR4A3 | 50-75 |
|             | t(9;17)(q22;q11)        | TAF2N-NR4A3   | 15-20        |
|             | t(9;15)(q22;q21)        | TFC12-NR4A3   | *            |
|             | t(9;22)(q22;q11)        | EWSR1-NR4A3   | *            |
|             | t(9;17)(q22;q11)        | TAF15-NR4A3   | *            |
|             | t(9;15)(q22;q21)        | TCF2-NR4A3    | *            |
|             | t(9;22)(q22;q15)        | TFG-NR4A3     | *            |
|             | t(12;22)(q13;q21)       | EWSR1-CHOP    | *            |
| Hemosiderotic fibrolipomatous tumour | t(1;10)(p22;q24) | TGFBR3-MGEA5 | 70-85 |
| Exraskeletal myxoid chondrosarcoma | t(9;22)(q22;q12) | EWSR1-NR4A3 | 50-75 |
|             | t(9;17)(q22;q11)        | TAF2N-NR4A3   | 15-20        |
|             | t(9;15)(q22;q21)        | TFC12-NR4A3   | *            |
|             | t(9;22)(q22;q11)        | EWSR1-NR4A3   | *            |
|             | t(9;17)(q22;q11)        | TAF15-NR4A3   | *            |
|             | t(9;22)(q22;q15)        | TFG-NR4A3     | *            |
|             | t(12;22)(q13;q21)       | EWSR1-CHOP    | *            |
| Alveolar soft part sarcoma | t(8;17)(p11;q25) | ASPSCR1-TFE3 | 80-95 |
| Myoepithelioma/myoepithelial carcinoma/mixed tumour | t(6;22)(p21;q12) | EWSR1-POUF1 | 23 |
|             | t(1;22)(q23;q12)        | EWSR1-PBX1    | 16           |
|             | t(1;16)(p34;p11)        | FUS-KLF17     | 9            |
|             | t(9;22)(q33;q21)        | EWSR1-PBX3    | 8            |
|             | t(12;22)(q13;q12)       | EWSR1-ZNF444  | *            |
|             | t(1;16)(p34;p11)        | FUS-KLF17     | 9            |
|             | t(6;10)(p21;q11)        | EPC1-PHF1     | *            |
|             | t(12;16)(q11;p13)       | ZC3H7B-BCOR   | *            |
| Soft tissue angiofibroma | t(5;8)(p15;q13) | AHRR-NCOA2 | 30-50 |
| Ossifying fibromyxoid tumour | t(6;12)(p24;q24) | EP400-PHF1 | 50-80 |
|             | t(1;6)(p34;p21)         | MEAF6-PHF1    | *            |
|             | t(6;10)(p21;q11)        | EPC1-PHF1     | *            |
|             | t(2;22)(p11;q13)        | ZC3H7B-BCOR   | *            |
| Clear cell sarcoma of soft tissue | t(12;22)(q13;q12) | EWSR1-ATF1 | 50-75 |
|             | t(2;22)(p13;q12)        | EWSR1-CREB1   | 6-13         |
|             | t(12;16)(q13;p11)       | FUS-ATF1      | *            |
### Table 1. Continued

| Tumour Type                                      | Chromosomal Translocation | Genes Involved     | Frequency (%) |
|--------------------------------------------------|---------------------------|-------------------|--------------|
| Clear cell sarcoma of the kidney                 | t(10;17)(q22;p13)         | YWHAE-NUTM2E      | 13-25        |
| Clear cell sarcoma like tumour of GI tract       | t(12;22)(q13;q12)         | EWSR1-ATF1        | 85-90        |
| Desmoplastic small round cell tumour             | t(2;22)(q33;q12)          | EWSR1-CREB1       | 6-20         |
| Desmoplastic small round cell tumour             | t(11;22)(p13;q12)         | EWSR1-WT1         | 90-99        |
| Desmoplastic small round cell tumour             | t(21;22)(q22;q12)         | EWSR1-ERG         | *            |
| Extraskeletal Ewing sarcoma                      | t(11;22)(q24;q12)         | EWSR1-FLI1        | 80-90        |
| Extraskeletal Ewing sarcoma                      | t(17;22)(q12;q12)         | EWSR1-EIAF        | 5-10         |
| Extraskeletal Ewing sarcoma                      | t(21;22)(q22;q12)         | EWSR1-ERG         | *            |
| Extraskeletal Ewing sarcoma                      | t(7;22)(q22;q12)          | EWSR1-ETV1        | *            |
| Extraskeletal Ewing sarcoma                      | t(2;22)(q36;q12)          | EWSR1-FEV         | *            |
| Extraskeletal Ewing sarcoma                      | t(16;21)(p11;q22)         | FUS-ERG           | *            |
| Extraskeletal Ewing sarcoma                      | t(2;16)(q36;p11)          | FUS-FEV           | *            |
| Intimal sarcoma                                  | amp(12)(q13-14)           | CDK4, TSPAN31, MDM2, G1  | 75-100      |
| Intimal sarcoma                                  | t(2;22)(q33;q12)          | EWSR1-CREB1       | 70-90        |
| Primary pulmonary myxoid sarcoma                 | t(2;22)(q33;q12)          | EWSR1-CREB1       | 70-90        |
| Primary pulmonary myxoid sarcoma                 | t(12;22)(q13;q12)         | EWSR1-ATF1        | 85-90        |
| Primary pulmonary myxoid sarcoma                 | t(2;22)(q36;q12)          | EWSR1-CREB1       | 6-20         |
| Primary pulmonary myxoid sarcoma                 | t(16;22)(q12;q12)         | EWSR1-ETV1        | *            |
| Primary pulmonary myxoid sarcoma                 | t(2;22)(q36;q12)          | EWSR1-FEV         | *            |
| Primary pulmonary myxoid sarcoma                 | t(16;22)(q12;q12)         | EWSR1-ETV1        | *            |
| PEComa                                           | t(X;17)(p11;p13)          | DVL2-TFE3         | *            |
| PEComa                                           | t(X; 1)(p11;p34)          | SFPO-TFE3         | *            |
| PEComa                                           | inv(X)(p11.2q12)          | NONO-TFE3         | *            |
| PEComa                                           | t(5;8)(q32;q24)           | HTR4-ST3GAL1      | *            |
| PEComa                                           | t(3;3)(p21;p13)           | RASSF1-PDZRN3     | *            |
| PEComa                                           | t(14;14)(q12;q24.1)       | RAD51B-RRAGB      | *            |
| Synovial sarcoma                                 | t(X;18)(p11;q11)          | SYT-SSX1          | 65-80        |
| Synovial sarcoma                                 | t(X;18)(p11;q11)          | SYT-SSX2          | 31-35        |
| Synovial sarcoma                                 | t(X;18)(p11;q13)          | SYT-SSX4          | *            |
| Synovial sarcoma                                 | t(X;20)(p11;q13)          | SS18L1-SSX1       | *            |
| Undifferentiated/unclassified sarcomas            | t(4;19)(q35;q13.1)        | CIC-DUX4          | 28-30        |
| Undifferentiated/unclassified sarcomas            | inv(X)(p11.2p11.4)        | BCOR-CCNB3        | 3-12         |
| Undifferentiated/unclassified sarcomas            | t(20;22)(q13;q12)         | EWSR1-NFATc2      | *            |
| Undifferentiated/unclassified sarcomas            | t(6;22)(p21;q12)          | EWSR1-POU5F1      | *            |
| Undifferentiated/unclassified sarcomas            | t(4;22)(q31;q12)          | EWSR1-SMARCAS5    | *            |
| Undifferentiated/unclassified sarcomas            | inv(22)(q12q12)           | EWSR1-PATZ1       | *            |
| Undifferentiated/unclassified sarcomas            | t(4;22)(q31;q12)          | EWSR1-SMARCAS5    | *            |
| Undifferentiated/unclassified sarcomas            | t(1;22)(p36;q12)          | EWSR1- ZSG        | *            |
| Undifferentiated/unclassified sarcomas            | t(2;22)(q31;q12)          | EWSR1-SP3         | *            |
| Undifferentiated/unclassified sarcomas            | t(10;19)(q26;q13)         | CIC-DUX10         | *            |
| Undifferentiated/unclassified sarcomas            | t(X;19)(q13;q13)          | CIC-FOXO4         | *            |
| Undifferentiated/unclassified sarcomas            | t(16;21)(p11;q22)         | FUS-ERG           | *            |
| Undifferentiated/unclassified sarcomas            | t(20;16)(q13;p11)         | FUS-NCATc2        | *            |
| Undifferentiated/unclassified sarcomas            | inv(X)(p11.4p11.22)       | BCOR-CCNB3        | *            |
| Undifferentiated/unclassified sarcomas            | t(12;15)(p13;q25)         | ETV6-NTRK3        | *            |
| Aneurysmal bone cyst                             | t(16;17)(q22;p13)         | CDH11-USP6        | 66-72        |

Data Sources: [1](http://cancer.sanger.ac.uk/cosmic); [2](https://www.mycancergenome.org); [3](http://www.genenames.org); [4](http://atlasgeneticsoncology.org); [5](http://cgap.nci.nih.gov/chromosomes); [6](http://www.genecards.org); [7](www.tumourfusions.org).

*Too infrequent to estimate percentage.

*limited reports.
is identifiable in more than 95% of cases. Commonly used FISH probes are included in the Vysis DDIT3 dual colour, break-apart rearrangement probe kit.

**Synovial sarcoma**

Synovial sarcoma is characterized by t(X;18)(p11;q11) translocation, which is present in virtually all tumours. The translocation fuses synovial sarcoma translocation gene (SS18) on chromosome 18 with either synovial sarcoma X breakpoint 1 (SSX1) (66%) or SSX2 (33%), located on chromosome Xp11 [32,33]. The FISH test is highly specific and sensitive for synovial sarcoma. A characteristic t(X;18)(p11;q11) translocation or variants were found in 90% of synovial sarcomas. The t(X;18) translocation has not been found in other sarcomas.

**Solitary fibrous tumour**

Solitary fibrous tumour comprises a family of soft tissue lesions usually affecting adults, occurring at any site, and presumed to be of fibroblastic differentiation. The tumours were thought to derive from pericytes, but now there is evidence to support a fibroblastic or myofibroblastic origin. Recurrent fusions of the two genes, NGFI-A–binding protein 2 (NAB2) and signal transducer and activator of transcription 6 (STAT6), both located near chromosomal region 12q13, have been identified in solitary fibrous tumours. The NAB2-STAT6 fusion gene is derived from an inverted intra-chromosomal fusion of the NAB2 and STAT6 genes on 12q13 [34,35]. The fusion product contains the activation domain of STAT6 fused to the early growth response (EGR)-binding domain of NAB2 [36]. Overexpression of the NAB2-STAT6 fusion gene induces cell proliferation and activates expression of EGFR-responsive genes. Chmielecki et al identified the NAB2-STAT6 translocation by whole exome sequencing from 17 solitary fibrous tumours and matched blood [34]. This fusion gene was confirmed in 29 of 55 (55%) solitary fibrous tumours by exome sequencing. Since the NAB2 and STAT6 genes are both located near 12q13, the break apart designed probe may detect some but not all of the NAB2-STAT6 translocations. Mohajeri et al used six independent molecular genetic techniques directed at NAB2 or STAT6 rearrangement to identify NAB2–STAT6 fusion in 37 of 41 (90%) of the solitary fibrous tumours evaluated [37].

It should be noted that the NAB2-STAT6 fusion is not consistently detected by FISH due to the very close proximity of the two genes [36] and, in this setting, reverse transcription polymerase chain reaction or RNA-Seq, is the preferred method of detection. STAT6 immunostaining is highly sensitive and specific for detecting NAB2-STAT6 fusion product and is now becoming standard practice.

**Round cell Ewing-like sarcoma**

There is a small subset of sarcomas that clinically and histologically mimic Ewing sarcoma but fail to exhibit any of the cytogenetic abnormalities reported for these tumours. Recently cases of ‘Ewing-like sarcoma’ have been found to have a recurrent chromosomal translocation, t(4;19)(q35;q13), resulting in fusion of capicua homologue (CIC) and double homeobox 4 (DUX4) [38]. Fusion of the C-terminal fragment of DUX4 with CIC enhances the transcriptional activity of CIC and deregulates expression of its downstream targets. CIC-DUX4 fusion protein directly binds the ETS variant 5 (ETV5) promoter at a previously unrecognized site and upregulates expression of this oncogene. Four cases of CIC-DUX4 sarcoma were identified using a combination of conventional cytogenetic, RT-PCR, and FISH methods [39]. FISH was positive for CIC-DUX4 fusion in all four tumours. The distinctive histopathological features and rapid disease progression may warrant classification of CIC-DUX4 sarcoma as a new translocation-associated sarcoma.

**Inflammatory myofibroblastic tumour**

Inflammatory myofibroblastic tumour is a spindle cell neoplasm most frequently found in the urinary bladder but occasionally diagnosed at various body sites. Although these tumours usually behave in a benign fashion, they may be difficult to differentiate from sarcoma or sarcomatoid carcinoma. Roughly half of inflammatory myofibroblastic tumours have ALK rearrangements leading to elevated expression of ALK chimeric protein [40]. The ALK translocations involve 2p23 with multiple fusion partners, including TPM3-ALK, RANBP2-ALK, TPM4-ALK, EML4-ALK, CLTC-ALK, CARS-ALK, ATIC-ALK, SEC31A-ALK and PPFIBP1-ALK [41,42]. In each instance, the fusion gene overexpresses ALK due to promoter swapping. ALK rearrangements may prove useful for distinguishing inflammatory myofibroblastic tumour from morphologically similar neoplasms.

Therapy targeting tumour cells with ALK gene rearrangement induces partial tumour remission [43,44], but, little is known about the pathogenesis of the 50% of inflammatory myofibroblastic tumours that lack ALK translocation.
Besides ALK, C-ROS oncogene 1 (ROS1) and NTRK3 gene rearrangements, YWHAE–ROS1 and ETV6–NTRK3 are also found in ALK fusion negative inflammatory myofibroblastic tumours [45]. Antonescu et al reported six (10%) ROS1 related translocations in a group of 62 cases. Most of the patients were children, and the tumours were located in the lung or abdomen [46]. Two (3.2%) inflammatory myofibroblastic tumour cases showed TFG-ROS1 fusions, t(3;6)(q12;q22).

Alveolar soft part sarcoma

Alveolar soft part sarcoma has a characteristic histopathology but a controversial histogenesis. Alveolar soft part sarcomas arising in many body sites have a diagnostic translocation. The rearrangement involves the alveolar soft part sarcoma locus (ASPSCR1) located on chromosome 17q25 and the transcription factor for the immunoglobulin heavy chain enhancer 3 (TFE3) gene, located on chromosome Xp11 [47]. The fusion protein is capable of inducing aberrant transcription of TFE3-regulated genes and may confer resistance to cell-cycle arrest signals and override apoptosis. Selvarajah et al investigated 17 alveolar soft part sarcomas from 11 patients by array comparative genomic hybridization and FISH. FISH identified the ASPSCR1-TFE3 fusion in all cases [12]. The ASPSCR1-TFE3 [der(17)t(X;17)(p11;q25)] translocation is found in 80% of alveolar soft part sarcomas. This translocation is also found in a distinctive subset of renal cell carcinoma (translocation renal cell carcinoma) which frequently has a papillary architecture.

Alveolar rhabdomyosarcoma

Alveolar rhabdomyosarcoma (ARMS) is a highly aggressive soft tissue sarcoma associated with translocations involving PAX3-FOXO1 [t(2;13)(q35;q14)] or PAX7-FOXO1 [t(1;13)(p36;q14)] reportedly accounting for 55–80% and 15–22% of ARMS, respectively [48]. These translocations generate fusion proteins that function as transcriptional activators with oncogenic effects. FISH testing has higher sensitivity and specificity than RT-PCR assay for these fusion transcripts [49]. FISH analysis using the FOXO1 split-apart probe adds the ability to detect variant FOXO1 rearrangements not detectable by PCR.

Clear cell sarcoma of soft tissue

Clear cell sarcoma of soft tissue is a rare mesenchymal malignancy mainly occurring in young to middle-aged adults of either sex, typically in the soft tissue of the lower extremities. The tumour is aggressive with a high frequency of local recurrence and distant metastasis. Almost all (90%) clear cell sarcomas are associated with EWSR1–ATF1 translocation, t(12;22)(q13;q12), while a smaller subset of tumours (6%) bear an EWSR1–CREB1 translocation [50]. The specific translocation is unrelated to tumour prognosis. In a group of 33 clear cell sarcomas, RT-PCR using RNA extracted from formalin-fixed, paraffin-embedded tissues demonstrated transcripts of the EWSR1–ATF1 (31/33) or EWSR1–CREB1 fusion gene (2/33) [51].

Chromosomal translocations in other solid tumours

A growing number of fusion oncogenes have been also associated with some common adult epithelial tumors, such as adenocarcinomas of the lung, prostate, colon, kidney, breast and other epithelial solid tumours (Table 2).

Chromosomal translocations mainly associated with lung cancer

EML4–ALK translocation. ALK is a tyrosine kinase member of the insulin receptor superfamily normally expressed only in certain neurons of the developing central nervous system. Fusions of ALK with echinoderm microtubule-associated protein-like 4 (EML4), a protein involved in microtubule assembly, results in constitutive activation of the ALK kinase [52,53]. The ALK fusion oncogene was first identified in anaplastic large-cell lymphoma, in which a t(2;5) chromosome rearrangement activates the ALK kinase by fusion with the nucleophosmin gene (NPM1) on chromosome 5. ALK fusions have been reported in non-small cell lung carcinoma, breast cancer, colorectal cancer, renal cancer and other tumour types [41,42,54,55]. The common FISH test for ALK rearrangement uses dual colour labelled probes covering the ALK gene and 3′ flanking region of ALK (Figure 3). The year 2013 was the first time that the FDA simultaneously approved a novel anticancer drug (crizotinib, Pfizer) and its companion FISH detection kit (ALK FISH probe kit, Abbott Molecular), highlighting the critical role of FISH triage for guiding ALK-targeted therapy [56,57]. A FDA approved Vysis ALK break apart FISH probe kit is recommended by the College of American Pathologists (CAP). In general, a sample is considered positive if >15% of cells
Table 2. Chromosome alterations in solid tumours of epithelial origin

| Tumour Type                        | Chromosome alteration | Gene Involved      | Frequency (%) |
|------------------------------------|-----------------------|--------------------|---------------|
| **Head and Neck/Salivary gland**   |                       |                    |               |
| Pleomorphic adenoma                | t(3;8)(p21;q12)       | CTNNB1–PLAG1       |               |
|                                    | t(5;8)(p13;q12)       | LIRF–PLAG1         |               |
|                                    | t(8)(p12;q12)         | FGFR1–PLAG1        |               |
|                                    | t(8;8)(q12;q12)       | CHCHD7–PLAG1       | 30–40         |
|                                    | t(8;8)(q11;q12)       | TCEA1–PLAG1        |               |
|                                    | t(8;8)(q21;q12)       | COL1A2–PLAG1       |               |
|                                    | t(8;8)(q24;q12)       | HAS2–PLAG1         |               |
|                                    | t(9;12)(p22;q14)      | HMGA2–NFIB         | 5–8           |
|                                    | t(3;12)(p14;q14)      | HMGA2–FHIT         |               |
| Adenoid cystic carcinoma           | t(6;9)(q22;p23)       | MYB–NFIB           | 30–50         |
| Clear cell odontogenic carcinomas  | t(22;12)(q12;q13)     | EWSR1–ATF1         | 63–82         |
| Hyalinizing clear cell carcinoma   | t(22;12)(q12;q13)     | EWSR1–ATF1         | 60–85         |
| Mucoepidermoid carcinoma           | t(11;19)(q21;p13)     | CRTC1–MAML2        | 50–70         |
|                                    | t(15;11)(q26;q21)     | CRTC3–MAML2        | 2–6           |
|                                    | t(1;11)(q21;q21)      | CRTC2–MAML2        | *             |
|                                    | t(6;22)(p12;q12)      | EWSR1–POUSF1       | *             |
| Mammary analogue secretory carcinoma | t(12;15)(p13;q25)    | ETV6–NTRK3         | 85–94         |
| NUT midline carcinoma              | t(15;19)(q13;p13)     | BRD4–NUTM1         | 70–80         |
|                                    | t(15;9)(q13;q34)      | BRD3–NUTM1         | 10–15         |
| Salivary duct carcinoma            | del(10)(q23)          | PTEN               | 40–50         |
| Solitary dermal cylindroma         | t(12;8)(q14;q12)      | HMGA2–PLAG1        | 22–50         |
| Squamous cell carcinoma of the head and neck | Amp(8)(p11) | FGFR1 | 9–15 |
| **Thyroid gland**                  |                       |                    |               |
| Papillary thyroid carcinoma        | inv(10)(q11q21)       | RET–CCDC6(PTC1)    | 60–70         |
|                                    | inv(10)(q11q10)       | RET–NCOA4(PTC3)    | 22–30\(^a\)  |
|                                    | t(1;15)(q21;q25)      | INTRK1–TPM3        | 12            |
|                                    | t(10;17)(q11;q23)     | RET–PRKAR1A(PTC2)  | 5–11\(^a\)   |
|                                    | t(12;15)(p13;q25)     | ETV6–NTRK3\(^a\)  | 2–14\(^a\)   |
|                                    | t(10;14)(q11;q32)     | RET–GOLGA5         | *             |
|                                    | t(7;10)(q32;q11)      | RET–TRIM24         | *             |
|                                    | t(10;14)(q11;q32)     | RET–TRIM27         | *             |
|                                    | t(1;10)(p13;q11)      | RET–TRIM33         | *             |
|                                    | t(10;14)(q11;q22)     | RET–KTN1           | *             |
|                                    | t(8;10)(p21;q11)      | RET–PCM1           | *             |
|                                    | t(6;10)(p21;q11)      | RET–TRIM27         | *             |
|                                    | t(8;10)(p11;q11)      | RET–Hook3          | *             |
|                                    | t(10;12)(q11;p13)     | RET–ERC1           | *             |
|                                    | t(10;18)(q11;q21)     | RET–MBD1           | *             |
|                                    | t(2;2)(p22;p23)       | STRN–ALK           | *             |
|                                    | t(7;7)(q21;q34)       | AKAP9–BRAF         | *             |
| Follicular thyroid carcinoma       | t(2;3)(q13;p25)       | PAX8–PPAR\(^\gamma\) | 30–35 |
| Tumour Type                        | Chromosome alteration | Gene Involved       | Frequency (%) |
|------------------------------------|-----------------------|---------------------|---------------|
| **Lung**                           |                       |                     |               |
| Lung adenocarcinoma                | inv(2)(p21p23)        | EML4-ALK            | 3–7           |
|                                   | t(2;3)(p23;q12)       | TFG-ALK             |               |
|                                   | t(2;10)(p23;p11)      | KIF5B-ALK           |               |
|                                   | t(2;14)(p23;q32)      | KL1C1-ALK           |               |
|                                   | t(2;9)(p23;q31)       | PTPN3-ALK           | 1–2           |
|                                   | t(2;22)(p23;p22)      | STRN-ALK            |               |
|                                   | t(2;4)(p23;q21)       | SEC31A-ALK          | 1–2           |
|                                   | t(2;7)(p23;q11)       | HIP1-ALK            |               |
|                                   | t(6;10)(q22;q21)      | CCDC6-ROS1          |               |
|                                   | t(6;7)(q22;p22)       | KDEL2-ROS1          |               |
|                                   | t(6;12)(q22;q14)      | LGIG3-ROS1          |               |
|                                   | t(6;20)(q22;q13)      | SDC5-ROS1           |               |
|                                   | t(6;5)(q22;q32)       | CD74-ROS1           | 2–3           |
|                                   | t(6;19)(q22;q13)      | FIG-ROS1            |               |
|                                   | t(6;6)(q22;q25)       | EZR-ROS1            |               |
|                                   | t(6;1)(q22;q21)       | TPM3-ROS1           |               |
|                                   | t(4;8)(p15;q22)       | SLC34A2-ROS1        |               |
|                                   | t(1;17)(q23;p11)      | MPRIIP-NTRK1        |               |
|                                   | t(1;5)(q23;q33)       | CD74-NTRK1          |               |
|                                   | inv(1)(q23q21)        | TPM3-NTRK1          | 1–3           |
|                                   | t(10;10)(q11;p11)     | KIF5B-RET           |               |
|                                   | t(10;10)(q11;q21)     | CCDC6-RET           |               |
|                                   | t(10;10)(q11;q11)     | NCOA4-RET           |               |
|                                   | t(1;10)(p13;q11)      | TRIM33-RET          | 1             |
|                                   |                       | TRIM24-NTRK1        | #             |
| **Primary pulmonary myxoid sarcoma** |                       | EWSR1-CREB1         | 70–90         |
| **Pleura and peritoneum**          |                       |                     |               |
| Mesothelioma                       | t(14;22)(q23;q12)     | EWSR1-YY1           | *             |
| **Gastrointestinal tract**         |                       |                     |               |
| Colorectal carcinoma               | t(6;6)(q22;q22)       | PTPRK-RSPO3         | 5–8           |
|                                   | t(11;21)(p15q11)      | EF3E-RSPO2          | 3–4           |
|                                   | t(2;2)(p23;p23)       | WDCCP-ALK           |               |
|                                   | inv(2)(p21p23)        | EML4-ALK            |               |
|                                   | t(4;6)(p15;q22)       | SLC34A2-ROS1        | 0.4–0.8       |
|                                   | t(10;10)(q25;q25)     | NAV2-TCF7L1         | *             |
|                                   | t(8;8)(p23;q23)       | V11A-TCF7L2         | *             |
|                                   | t(1;11)(q21;q23)      | NTRK1-TPM3          | *             |
| **Oesophageal adenocarcinoma**     | t(3;8)(p14.2;q24)     | FHIT-RNF139         | #             |
| Fibrolamellar hepatocellular carcinoma | t(19;19)(p13.1;p13.2) | DNAJB1-PRKACA      | 92–100        |
| **Intrahepatic cholangiocarcinoma** | t(1;1)(q23;q25)      | RABGAP15-NTRK1      | *             |
| **Gynaecological tract**           |                       |                     |               |
| Endometrial stromal sarcoma        | t(7;17)(p15q11)       | JAZF1-SUZ12         | 40–50         |
|                                   | t(7;10)(p13q23)       | YWAHE-NUTM2A        | 20–30         |
|                                   | t(7;10)(p13q23)       | YWAHE-NUTM2B        |               |
|                                   | t(1;6)(p34p21)        | MEAF6-PHF1          | 6–10          |
|                                   | t(6;7)(p21p15)        | JAZF1-PHF1          |               |
|                                   | t(6;10)(p21p11)       | EPC1-PHF1           |               |
| Ovarian papillary cystadenocarcinoma | t(6;14)(q21;q24)     | -                   | 32–40         |
Table 2. Continued

| Tumour Type                        | Chromosome alteration       | Gene Involved              | Frequency (%) |
|------------------------------------|-----------------------------|----------------------------|---------------|
| **Breast**                         |                             |                            |               |
| Secretory breast carcinoma         | t(12;15)(p13;q25)           | ETV6–NTRK3, TENM4–NRG1      | 56–100        |
|                                    | t(8;11)(p12;q14)            |                            | *             |
|                                    | t(3;8)(q26;q25)             | TBL1X1R1–RG517             | *             |
|                                    | dic(8;11)(p12;q14)          | TENM4–NRG1                 | *             |
| Triple-negative breast carcinoma   | t(1;1)(p13;q44)             | MAGI3–AKT3                 | 1             |

**The urinary system and male genital organs**

| Oncocytoma                         | t(5;11)(q35;q13)             | -                          | *             |
| Metanephric adenoma                | t(6;9)(p12;p24)              | -                          | *             |
|                                    | t(1;22)(q22;q13)             | EWSR1–PBX1, TENM4–NRG1    | *             |
|                                    | t(1;5;16)(q21;p13)           | -                          | *             |
|                                    | t(1;1)(p13;q44)              | MAGI3–AKT3                 | 1             |

**Clear cell renal cell carcinoma**

| del(3)(p11–pter)                   | VHL, PBRM1, BAP1, SETD2     | 70–90                      |
| del(14)(q23)                       | HIF1A                       | 35–40                      |
| Papillary renal cell carcinoma     | + 7                         | MET                        | 80            |
|                                   | + 17                        | MET                        | 85            |
|                                   | -Y                          | -                          | 70            |
| Renal carcinomas associated with Xp11.2 translocations | t(X;1)(p11.2;q21) | PRCC–TFE3 | 60–70 |
|                                    | t(X;1)(p11.2;p34)            | SFPQ–TFE3                  | 10–20         |
|                                    | t(X;17)(p11.2;q25)           | ASPSCR1–TFE3, NONO–TFE3   | *             |
|                                    | t(X;17)(p11.2;q23)           | CLTC–TFE3, LUC7L3–TFE3    | *             |
|                                    | t(X;19)(p11.2;p13.3)         | KHSRP–TFE3                 | *             |
| Renal cell carcinoma associated with t(6;11) translocations | t(6;11)(p21;1;q12) | MALAT1–TFEB | 90–100 |
|                                    | t(6;6)(p21;1;q11)            | KHDRBS2–TFEB              | *             |
| Chromophobe renal cell carcinoma   | - 1                         | -                          |               |
|                                    | - 2                         | -                          |               |
|                                    | - 6                         | -                          |               |
|                                    | - 10                        | -                          | 70–90         |
|                                    | - 13                        | -                          |               |
|                                    | - 17                        | -                          |               |
|                                    | - 21                        | -                          |               |
| Renal medullary carcinoma          | t(1;22)(q41;q11)            | SMARCB1–CAPN2              | *             |
|                                    | t(15;22)(q22;q11)            | SMARCB1–RORA               | *             |
|                                    | t(11;22)(q21;q11)            | SMARCB1–MAML2              | *             |
|                                    | t(4;7)(p16;q22)              | FGFR3–BAIAP2L              | 5–9           |
| ALK translocation associate renal cell carcinoma | t(2;1)(p23;q21) | TPM3–ALK | 100 |
|                                    | t(2;10)(p23;q22)             | VCL–ALK                    | *             |
| Wilms tumour                       | i(17)(q10)                  | YWHAE–NUTM2E               | 13–25         |
|                                    | i(7)(q10)                   | -                          | *             |
| Clear cell sarcoma of the kidney   | t(10;17)(q22;p13)           | YWHAE–NUTM2E               | 13–25         |
| Urothelial carcinoma               | Polysomy 3                  | -                          |               |
|                                    | Polysomy 7                  | -                          |               |
|                                    | Polysomy 17                 | -                          | 80–94         |
|                                    | del(9)(p21)                 | CDKN2A                     |               |
|                                    | t(4;7)(p16;q22)              | FGFR3–BAIAP2L              | 5–9           |
| Tumour Type                      | Chromosome alteration | Gene Involved     | Frequency (%) |
|---------------------------------|-----------------------|-------------------|---------------|
| Prostatic adenocarcinoma        | t(4;4)(p16;p16)       | FGFR3-TACC3       | 3-6           |
|                                 | t(12;16)(q13;p13)     | SCN8A-GRIN2A      | #             |
|                                 | t(21;21)(q22;q22)     | TMPRSS2-ERG       | 47-79         |
|                                 | t(21;16)(q22;q13)     | HERPUD1-ERG       |               |
|                                 | t(21;11)(q22;q32)     | SLCA45A3-ERG      |               |
|                                 | t(21;8)(q22;q24)      | NDRG1-ERG         |               |
|                                 | t(7;22)(p21;q22)      | ETV1-ERG          | *             |
|                                 | t(17;22)(q21;q22)     | ETV4-ERG          | *             |
|                                 | t(7;21)(p21;q22)      | TMPRSS2-ETV1      |               |
|                                 | t(7;1)(p21;q22)       | SLC45A3-ETV1      | *             |
|                                 | t(7;15)(p21;q21)      | C15orf21-ETV1     |               |
|                                 | t(7;7)(p21;q15)       | HRNPA2B1-ETV1     | 7-10          |
|                                 | t(7;11)(p21;q15)      | ASCL3-ETV1        |               |
|                                 | t(7;14)(p21;q13)      | EST14-ETV1        |               |
|                                 | t(7;17)(p21;q13)      | HERVH17-ETV1      |               |
|                                 | t(1;1)(q32;q32)       | TMPRSS2-ETV4      | *             |
|                                 | t(17;21)(q21;q22)     | CANT1-ETV4        | *             |
|                                 | t(17;17)(q21;q25)     | KLK2-ETV4         | *             |
|                                 | t(3;21)(q27;q22)      | TMPRSS2-ETV5      | *             |
| Testicular germ cell tumour     | del(10)(q23)          | PTEN              | 40-70         |
| Skin                            | i(12p)                |                  | 67-83         |
| Spitz nevi                      | t(5;6)(q33;q22)       | PWIWP2A-ROS1      |               |
|                                 | t(6;6)(p22;q22)       | HLA-A-ROS1        |               |
|                                 | t(6;12)(q22;p11)      | PPIBP1-ROS1       |               |
|                                 | t(6;15)(q22;q21)      | MYO5A-ROS1        |               |
|                                 | t(6;12)(q22;p13)      | ERC1-ROS1         |               |
|                                 | t(6;12)(q22;q24)      | CLIP1-ROS1        |               |
|                                 | t(6;12)(q22;q24)      | ZCCHC8-ROS1       |               |
|                                 | t(6;10)(q22;q25)      | SHT11-ROS1        |               |
|                                 | t(3;6)(q12;q22)       | TFG-ROS1          |               |
|                                 | t(2;2)(p23;p23)       | DCTN1-ALK         | 11-15         |
|                                 | t(1;2)(q23;p23)       | TPM3-ALK          |               |
|                                 | t(1;17)(q23;p13)      | TP53-NTRK1        | 7-16          |
|                                 | t(1;1)(q23;q22)       | LMNA-NTRK1        |               |
|                                 | t(10;14)(q11;q32)     | GOLGA5-RET        | 3             |
|                                 | t(10;10)(q11;p11)     | KIF5B-RET         |               |
|                                 | t(7;19)(q34;q13)      | CEP89-BRAF        | *             |
|                                 | t(7;19)(q34;q13)      | LSM14A-BRAF       | *             |
|                                 | t(2;7)(q37;q31)       | LRRFIP1-MET       | *             |
| Melanoma                        | amp(11)(q13)          | CCND1             | 54-82        |
|                                 | amp(6)(p25)           | RREB1             |               |
|                                 | amp(6)(q23)           | MYB               |               |
are positive for ALK separation of the green and orange signals.

**ROS1** translocation. **ROS1** is a receptor tyrosine kinase of the insulin receptor family. The **ROS1** rearrangements lead to a constitutively activated fusion kinase, and are detected in 1.2–1.7% of lung adenocarcinoma cases [58–60]. **ROS1** activation stimulates other downstream signalling proteins including AKT1, MAPK1, MAPK3, IRS1 and PLCG2 [59]. Patients with **ROS1** translocation are significantly

| Tumour Type          | Chromosome alteration | Gene Involved | Frequency (%) |
|----------------------|-----------------------|---------------|---------------|
| Glioblastomas        | del(10)(q23)          | PTEN          | 43            |
|                      | t(7;7)(q31;q31)       | PTPRZ1-MET    | 15-35         |
|                      | t(4;4)(p16;p16)       | FGFR3-TACC3   | 5-8           |
|                      | t(4;8)(p16;p11)       | FGFR1-TACC3   |               |
|                      | t(3;7)(p27;q7)        | ?-BCL6        | 5             |
|                      | t(7;7)(p11;p12)       | LANCL2-SEPT14 | *             |
| Angiocentric glioma  | t(6;6)(q23;q26)       | MYB-OK1       | 80            |
| Pilocytic astrocytoma| t(7;7)(q34;q34)       | KIAA1549-BRAF | 64-73         |
|                      | t(7;5)(q34;q35)       | RNF130-BRAF   |               |
|                      | t(9;6)(q21;p26)       | QKI-NTRK2     | *             |
| Medulloblastosma     | i(17q)                | E2A-PBX1      | 30-50         |
|                      | t(1;19)(q23;p13)      | -             | #             |
| Meningioma           | del(22)(q12)          | NF2           | 50-60         |
|                      | t(12;22)(p13;q12)     | MNI-ETV6      | #             |
|                      | t(1;19)(q21;q13)      | -             | #             |
| Ependymoma           | -del(22)(q11)         | -             | 20-30         |
|                      | t(11;11)(q13;q13)     | C11orf95-RELA | 71-80         |
|                      | t(2;14)(p23;q22)      | KTV1-ALK      | #             |
|                      | del(2)(p16p23)        | CCDC88A-ALK   | #             |
| Extraventricular neurocytoma | t(1;19)(q10;p10)    | -             | 17-20         |
| Papillary glioneuronal tumour | t(9;17)(q31;q24) | SLC44A1-PRKCA | #             |

Data Sources: [http://cancer.sanger.ac.uk/cosmic](http://cancer.sanger.ac.uk/cosmic); [https://www.mycancergenome.org](https://www.mycancergenome.org); [http://www.genenames.org](http://www.genenames.org); [http://atlasgeneticsoncology.org](http://atlasgeneticsoncology.org); [http://cgap.nci.nih.gov/chromosomes](http://cgap.nci.nih.gov/chromosomes); [http://www.genecards.org](http://www.genecards.org); [www.tumourfusions.org](http://www.tumourfusions.org).

*Too infrequent to estimate percentage.

*Limited report.

*Higher incidence in radiation induced thyroid cancers.

*50% with sickle-cell trait.

The UroVysion Bladder Cancer Kit (UroVysion Kit) is FDA approved and designed to detect aneuploidy for chromosomes 3, 7, 17, and loss of the 9p21 locus in urothelial tumours.

The Vysis Melanoma FISH Probe Kit is designed to detect copy number of the RREB1 (6p25), MYB (6q23), CCND1 (11q13) genes and of centromere 6 in melanocytic lesions.
younger than most patients with lung cancer and are more likely to be never-smokers. Their cancers tend to be adenocarcinoma, and their tumours have a tendency towards higher grade. Several genes may serve as fusion partners for ROS1 including SDC4, SLC34A2, CD74, GOPC, and EZR, yet each of the ROS1 fusion products leads to constitutive ROS kinase activation [61]. A dual probe break-apart method is used to detect ROS1 rearrangement as with ALK rearrangement. Criteria similar to those used for ALK rearrangement screening are used to evaluate the ROS1 FISH test.

ROS1 fusions are also found in meningioma, cholangiocarcinoma and glioblastoma, but no systematic investigation of tyrosine kinase inhibitors has been published for ROS1-fusion positive tumours of these primary sites [62,63]. The reported incidence of ROS1 translocation is also low in other tumour types: 8.7% (2/23) of cholangiocarcinomas, 0.5% (1/200) of ovarian carcinomas, 0.6% (3/495) of gastric adenocarcinomas, 0.8% (2/236) of colorectal carcinomas, 7.7% (2/26) of inflammatory myofibroblastic tumours, 2.9% (1/34) of angiosarcomas and 5% (1/20) of epithelioid haemangioendotheliomas [63–66].

**Rearranged during transfection translocation.** Novel chromosomal translocations involving the rearranged during transfection (RET) tyrosine kinase gene were reported recently [61,67]. The RET gene belongs to the cadherin superfamily, and encodes a cell surface receptor tyrosine kinase that transduces signals for cell growth and differentiation [67,68]. Several RET rearrangements have been identified in non-small cell lung carcinomas, including KIF5B-RET, CCDC6-RET, NCOA4-RET, and TRIM33-RET.

RET gene translocation occurs in approximately 1–2% of non-small cell lung carcinomas and defines a clinically distinct subset of non-small cell lung carcinomas. Patients with adenocarcinomas with RET fusion tend to be younger than most patients with lung cancer and they are more likely to be never-smokers [69]. In a study of 936 patients with non-small cell lung carcinoma, the RET fusion gene was exclusively detected in 13 patients (11/633, 1.7% in adenocarcinoma; 2/24, 8.3% in adenosquamous cell carcinoma).
Of the 13 RET fusion-positive patients, 9 (69%) had KIF5B-RET, 3 (23%) had CCDC6-RET, and 1 (7.6%) had NCOA4-RET fusion. Their tumours tended to be poorly differentiated carcinomas that exhibited early lymph node metastasis. Drilon et al found RET fusions in 5 of 31 patients and showed that the RET kinase can be effectively inhibited by several small molecule inhibitors [71]. Follow-up imaging conducted after 4 and 12 weeks of therapy confirmed a partial response with a 66% decrease in measurable disease in the lungs and pleura.

**EWSR1-CREB1 translocation in primary pulmonary myxoid sarcomas.** In the 2015 World Health Organization (WHO) Classification of Tumours of the Lung, Pleura, Thymus and Heart, pulmonary myxoid sarcoma with EWSR1-CREB1 translocation was introduced as a new entity [72]. Primary pulmonary myxoid sarcoma is rare, and arises most frequently in young females. The tumour consists of lobules with delicate, lacelike strands and cords of mildly atypical round or spindle cells in a prominent myxoid stroma. In addition to displaying a distinct morphology, it bears a EWSR1-CREB1 translocation in 70% of cases [73]. It should be noted that EWSR1-CREB1 translocation is not unique for this entity; it can also be observed in clear cell sarcoma-like tumour of the gastrointestinal tract and angiomatoid fibrous histiocytoma (Table 1).

**Chromosomal translocations mainly associated with kidney cancer**

**TFE3 translocation**

Renal cell carcinomas associated with Xp11.2 translocation are uncommon renal tumours that were recognized as a distinct entity in the 2016 World Health Organization classification of Tumours of the Urinary System and Male Genital Organs [74]. These neoplasms comprise the majority of paediatric renal cell carcinomas and a smaller percentage of adult renal cell carcinomas. There are several different translocations involving chromosome Xp11.2, resulting in gene fusions of the TFE3 gene with various activating partners. At least five different fusion partners for TFE3 have been characterized, including ASPSCR1, PRCC, SFPQ, CLTC and NONO (Figure 4) [75,76]. Variant translocations with unknown fusion partners include t(X;3)(p11.2;q23) and t(X;10)(11.2;q23). Different gene fusions in Xp11.2 translocation renal cell carcinoma may be associated with different morphological features [77,78].

**Figure 4.** Genomic translocation relationships presented by Circos plot. Circos applies a circular ideogram displaying relationships between genomic intervals. The Circos plot is a ring composed of all chromosomes in proportionate size clockwise from 1 to Y. This graphic representation is used to visualize chromosomal translocations associated with a clinical entity. A specific translocation is shown with a line connecting the two chromosome partner loci involved in each translocation. The chromosomal translocations that may be involved in Xp11 translocation renal cell carcinomas are shown in A, including inv(X)(p11.2;q12) [NonO-TFE3] (Red), t(X;1)(p11.2;p34) [PSF-TFE3] (Gray), t(X;1)(p11.2;q21) [PRCC-TFE3] (Blue), t(X;3)(p11.2;q23) [unknown-TFE3] (Black), t(X;10)(p11.2;q23) [unknown-TFE3] (Yellow), t(X;17)(p11.2;q23) [CLTC-TFE3] (Magenta), t(X;17)(p11.2;q23) [APPL-TFE3] (Green), t(X;19)(p11.2;q13.1) [unknown-TFE3] (Purple). Translocations involved in TFEB translocation renal cell carcinoma are shown in B, t(6;11)(p21;q13) [MALAT1-TFE8] (Blue), t(6;17)(p21;q25) [ASPSCR1-TFE8] (Red).
The TFE3 break-apart FISH assay has proven useful for detecting TFE3 gene fusions in Xp11.2 translocation renal cell carcinoma, and the FISH assay, in comparison with immunohistochemical evaluation, is less often adversely affected by technical and fixation issues. Rao et al analysed 24 poorly differentiated renal cancers with break-apart TFE3 FISH [79]. Seventeen cases showed TFE3 rearrangement associated with Xp11.2 translocation by FISH, including seven previously unclassified renal cell tumours, supporting the diagnostic value and clinical application of FISH for enigmatic renal tumours. On the basis of commercially available and widely tested break-apart FISH assays, the TFE3 split-signal pattern was considered positive when ≥10% of the tumour nuclei showed separation of the two coloured signals by more than one signal diameter (Figure 5).

Transcription factor EB translocation

The t(6;11) translocation renal cell carcinoma is rare and usually bears a translocation between transcription factor EB (TFEB) and MALAT1 on chromosome 11q12, but rarely TFEB may partner with KHDRBS2 on chromosome 6q11 (Figure 4B) [80]. Renal cell carcinomas with t(6;11)(q21;ql2) are characterized by translocation involving TFEB. Identification of either TFEB protein overexpression or t(6;11) translocation is useful for the diagnosis of TFEB renal cell carcinoma. Cytogenetic karyotypic analysis and RT-PCR

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**Figure 5.** Signal patterns of TFE3 split-apart probes. Renal cell carcinomas associated with Xp11.2 translocation involve several different translocations resulting in gene fusions of the TFE3 gene with various fusion partners. The break-apart FISH assay uses probes both upstream (yellow) and downstream (red) to TFE3 showing different signal patterns in female (A) and male (B) patients. A positive result in a female patient shows a fused or closely approximated normal yellow-red signal pair (uninvolved X chromosome) and either a pair of split-apart signals or a single yellow or red signal due to section truncation artifact. Because males have only one X-chromosome, a positive result in a male patient consists either of a pair of split-apart yellow and red signals, or of a single yellow or red signal due to section truncation.

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are also common methodologies for identifying this translocation. Unfortunately, these methods are limited by the need for fresh viable tumour cells for culture or flash frozen tissue to quantitatively assay TFEB mRNA. More recently, a break-apart FISH assay to detect TFEB gene translocation has been validated on formalin-fixed paraffin-embedded tissues, and appears to be superior to the TFEB IHC assay. Rao et al reported seven patients with TFEB renal cell carcinoma confirmed diagnostically by using FISH technology. All seven cases were TFEB positive by both fusion and split-apart probe designs [81].

**SMARCB1/SMARCB1 genetic alterations and ALK translocation**

Renal medullary carcinoma is a rare, aggressive tumour with a poor clinical outcome. It belongs to a SMARCB1-deficient tumour family [82]. Inactivation of tumour suppressor gene SMARCB1 is the hallmark of this tumour. Cheng et al studied a total of 19 renal cancers that included 5 renal medullary carcinomas, 2 paediatric rhabdoid tumours of kidney, 10 high grade renal cell carcinomas and 2 urothelial carcinomas [83]. All renal medullary carcinomas were from African American patients with sickle-cell trait who presented with extensive extrarenal metastases at the time of diagnosis. All five renal medullary carcinomas and two renal rhabdoid tumours showed complete loss of SMARCB1 expression. Another study by Calderaro et al found recurrent alterations involving chromosome 22q11 in each of four renal medullary carcinomas associated with sickle-cell trait. Each of these showed hemizygous SMARCB1 deletion [84]. RNA sequencing further identified fusion transcripts involving SMARCB1 in these tumours. The transcripts resulted from balanced translocations disrupting SMARCB1 and fusing it to various partners including CAPN2, RORA, MAML2 and MALAT1.
ALK translocation was also reported in renal medullary carcinomas from young patients with sickle-cell trait to be associated with VCL-ALK translocation [85]. The incidence of ALK rearrangement associated with all renal cell carcinomas is low, with an overall frequency of <1%. In a study including 534 adult renal cell carcinoma patients, ALK rearrangements occurred in two papillary renal cell carcinomas [86]. More recently, Kusano et al. reported two cases of RCC harbouring a novel STRN-ALK fusion [87]. In both cases, the patients were in their 30s at the time of nephrectomy.

Chromosomal translocations mainly associated with prostate cancer

A significant event in prostate carcinogenesis involves gene fusion between members of the E-twenty-six transforming factor (ETS) family of genes, including ERG, and the transmembrane protease, serine 2 (TMPRSS2, 21q22.3) gene [88–91]. The genes involved are the androgen-regulated gene TMPRSS2 and ETS transcription factor family members, including ERG (21q22.2), ETV1 (7p21.2), or ETV4 (17q21). TMPRSS2-ERG gene fusion has been identified in about 50% of prostate tumours (ranging from 27% to 79%). These fusions appear to represent a specific early event in prostatic carcinogenesis that can be observed in high grade prostatic intraepithelial neoplasia [90]. The TMPRSS2-ERG rearrangement can be identified by both dual colour split apart probe or by tricolour probes (Figure 6) [92–94].

Chromosomal translocations mainly associated with breast cancer

A rare variant of breast cancer, secretory breast cancer, has a characteristic recurrent translocation between ETS variant 6 (ETV6) and neurotrophic tyrosine kinase type 3 (NTRK3), t(12;15)(p13;q25) [95,96]. This translocation appears to be the initial hit required for formation of secretory breast cancer. Retroviral transfer of ETV6-NTRK3 into murine mammary epithelial cells resulted in transformed cells that readily formed tumours in nude mice.

The biological consequence of this translocation is expression of a chimeric tyrosine kinase protein with potent transforming capability. The fusion protein strongly activates the MAPK1 and the PIK3CA/AKTI pathways [96]. The occurrence of the ETV6-NTRK3 translocation and consequential expression of the oncogene is an initiating event in the genesis of secretory breast cancer [97]. The ETV6-NTRK3 translocation is demonstrated either by break-apart or by fusion FISH design. Positive signal patterns have been seen in most secretory breast carcinoma tumour cells using either assay strategy.

Chromosomal translocations mainly associated with colorectal cancer

The first recurrent fusion gene in colorectal cancer was reported in 2011 [98]. Bass et al. correlated the genomes of nine colorectal cancers with paired nonneoplastic tissue controls. Recurrent V11A-TCF7L2 fusion was identified in 3% of colorectal cancers [98]. Their findings indicated that functionally important fusion events also occur in colorectal cancers, but the clinical impact of these is yet to be elucidated. RNA-sequencing studies reported by Seshagiri et al. identified multiple fusion transcripts in colorectal cancer, including recurrent gene fusions involving R-spondin family members EIF3E-RSPO2 and PTPRK-RSPO3 that occurred with a cumulative frequency of 10% in colorectal cancers [99]. Other translocations reported in recent years include EML4-ALK, C2orf44-ALK, SLC34A2-ROS1 and NAV2-TCF7L1 [66].

Chromosomal translocations mainly associated with thyroid cancer

More than 10 rearrangements involving RET have been reported; all of the rearrangements are formed by fusion of the RET tyrosine kinase domain with a fusion partner gene [100–103]. RET rearrangements have been identified almost exclusively in papillary thyroid carcinomas; most frequent were RET-CCDC6 (60%), RET-NCOR2 (20%) and RET-PRKAR1A (5–12%) [67,68,104]. The fusion genes code for constitutively activated proteins driving uncontrolled proliferation of follicular cells.

Kelly et al. reported that STRN-ALK fusion occurs in a subset of patients with highly aggressive types of thyroid cancer and provide initial evidence suggesting that it may represent a therapeutic target for these patients. STRN-ALK fusion protein leads to constitutive activation of ALK kinase [105].

Follicular thyroid carcinoma accounts for approximately 20% of all thyroid malignancies. The t(2;3)(q13;p25) translocation, fusion of the thyroid
transcription factor, paired box gene 8 (PAX8) with peroxisome proliferator activated receptor γ (PPARG) is most commonly observed (30%) in follicular thyroid carcinoma [106]. The fusion product acts as an oncogene by accelerating cellular growth, down-regulating apoptosis, promoting anchorage-independence, and causing loss of contact inhibition. It is noteworthy that rearrangement of these genes has also been identified in benign thyroid adenomas with a break-apart frequency between 3% and 6% [107,108].

Chromosomal translocations mainly associated with salivary gland tumours

Approximately 20% of salivary gland tumours harbour chromosomal translocation [109]. There are four major recurrent translocations in malignant salivary gland tumours: the MYB-NFIB fusion in adenoid cystic carcinoma, the CRTC1-MAML2 fusion in mucoepidermoid carcinoma, the ETV6-NTRK3 fusion in mammary analogue secretory carcinoma (MASC), and the EWSR1-ATF1 fusion in hyalinizing clear cell carcinoma (HCCC) (Table 2) [96,97,110,111]. The identification of recurrent tumour-specific chromosomal translocations and novel fusion oncogenes has diagnostic, therapeutic, and prognostic implications.

Pleomorphic adenoma

Pleomorphic adenoma is a benign salivary gland tumour most often arising in the parotid gland. The tumour presents with a highly specific and recurrent pattern of chromosome abnormalities in 70% of cases. There are four cytogenetic categories of pleomorphic adenoma. These involve 8q12 (39%), 12q13-15 (8%), other recurring translocations (23%), and cytogenetically unchanged cases (30%). Of cases with rearrangements, the 8q12 rearrangement [t(3;8)(p21;q12)], represents almost half of the diagnostic translocations found.

Mucoepidermoid carcinoma

Mucoepidermoid carcinoma represents a distinct type of polymorphous tumour, containing three cellular elements in varying proportions: squamous cells, mucus-secreting cells, and ‘intermediate’ cells. The CRTC1-MAML2 fusion is present in 55% of mucoepidermoid carcinomas. The fusion oncogene derived from chromosomal translocation that binds cyclic-AMP response element protein related transcription co-factor 1 (CRTC1) with mastermind-like 2 NOTCH signalling coactivator (MAML2), t(11;19)(q12;p13) is aetiological for some mucoepidermoid carcinomas. When present, this marker confers a favourable survival outcome when compared with fusion-negative tumours [112].

Adenoid cystic carcinoma

Recurrent t(6;9)(q22–23;p23–24) translocation in adenoid cystic carcinoma results in a fusion of the MYB proto-oncogene with transcription factor gene NFIB [113]. MYB is a leucine zipper transcription factor at 6q22-24 that participates in the regulation of cell proliferation, apoptosis, and differentiation [114]. West et al used FISH to investigate MYB translocation in 37 of these tumours. MYB-NFIB translocation was present in 49% of adenoid cystic carcinomas but not in other salivary gland tumours or non-salivary gland neoplasms [115]. A subset of about 20% of adenoid cyst carcinomas lacks any detectable MYB gene fusion [116,117]. The high frequency of this fusion in adenoid cystic carcinoma indicates that the transcript may play a developmental role in a significant subset of these tumours. Therapies directed against MYB-NFIB transcriptional targets may improve the prognosis for this chemoresistant neoplasm.

Mammary analogue secretory carcinoma

MASC is a recently recognized salivary gland tumour that is histologically, immunohistochemically, and genetically similar to secretory carcinoma of the breast [118]. MASC harbours a t(12;15) (p13;q25) translocation resulting in fusion of an E-twenty-six family member (ETV6) and the neurotrophic tyrosine kinase receptor type 3 (NTRK3) gene [119]. Histologically, conventional MASC displays a lobulated growth pattern of a tumour composed of microcystic, tubular, and solid structures. Between epithelial structures, one finds abundant eosinophilic homogenous or bubbly secretions. Majew ska et al investigated seven MASC by FISH, and the ETV6-NTRK3 rearrangement was identified in six cases [120]. For FISH analysis, a dual-colour break-apart probe for the ETV6 gene exhibits a ‘split’ fluorescent signal in nuclei where the ETV6 gene participates in translocation. MASC behaves as a low-grade carcinoma with 15–20% recurrence, lymph node metastases in 15–20% of cases, and occasional mortality [118,121]. ETV6 FISH is useful to diagnose difficult cases and to exclude the tumours that morphologically mimic MASC.
Hyalinizing clear cell carcinoma

HCCC is a recently described minor salivary gland tumour composed of glycogen-rich clear cells with hyalinized or myxoid stroma. Gene fusion between Ewing sarcoma breakpoint region 1 (EWSR1) and the activating transcription factor 1 (ATF1) gene is a relatively consistent finding in HCCC [122]. However, EWSR1-ATF1 translocation could also be found in other types of tumour including angiomatoid fibrous histiocytoma, angiosarcoma of the parotid gland, clear cell sarcoma of soft tissue, and clear cell sarcoma-like tumour from the GI tract [51,123–126]. In one study, the ATF1 gene translocation was found in 82% of HCCC cases, but in none of the other tumours in the differential diagnosis [109].

Chromosomal translocations mainly associated with Spitz nevi and melanoma

The genetic underpinnings of Spitz nevi are poorly understood, and alterations in melanoma-associated oncogenes are typically absent. Spitzoid neoplasms harbour kinase fusions of ROS1 (17%), NTRK1 (16%), ALK (10%), BRAF (5%) and RET (3%) in a mutually exclusive pattern [127]. The chimeric proteins are constitutively activated, which stimulate oncogenic signalling and initiate the tumorigenesis. In a study of 140 spitzoid neoplasms, Wiesner et al demonstrated that kinase fusions were present in 18 of 30 Spitz nevi (60%) and within 6 of 8 atypical Spitz tumours (75%). The kinase gene fusions involved ROS1 (26%, 16/73), ALK (11%, 8/75), NTRK1 (11%, 8/75), BRAF (5%, 4/75) and RET (3%, 2/75). FISH can be used as an ancillary tool in the diagnosis of ambiguous melanocyte neoplasms, since Spitz nevi are benign and do not require wide excision [127].

Molecular alterations commonly seen in melanomas are typically absent from Spitz nevi and Spitz nevi contain translocations are not usually found in melanoma [91,128–130]. Translocations involving BRAF and MET genes have been recently reported in melanoma patients (Table 2). BRAF fusion genes characteristically activate the MAPK pathway with transformation abilities [131,132]. This activation of the MAPK pathway renders the tumours potentially sensitive to MEK inhibition.

MET is a high-affinity tyrosine kinase receptor with functions in angiogenesis, cellular motility, growth and invasion [133]. Yeh et al analysed 1202 equivocal pigmented skin lesions representing spitzoid melanoma, Spitz nevi, conventional nevi, deep penetrating nevus-like lesions and blue nevi. The patients’ tumours were tested by comparative genomic hybridization. MET translocations were found in six melanocytic tumours resulting in the following fusion genes: TRIM4-MET, ZKSCAN1-MET, PPFIBP1-MET, LRRFIP1-MET, EPS15-MET and DCTN1-MET [134]. MET fusion genes are driven by the promoter of the partner gene and express MET without control.

Chromosomal translocations mainly associated with tumours of the central nervous system

The WHO 2016 classification of tumours of the central nervous system emphasizes the importance of molecular classification [135]. More than 10 translocations have been reported in glioblastomas and gene fusions occur in approximately 30–50% of glioblastoma patients (Table 2) [136–139]. PTPRZ1-MET translocation was detected in 15% of glioblastomas in independent cohorts, rendering it the most frequently recurring transcript in glioblastoma. Recently, Bao et al investigated 272 gliomas by RNA seq and identified 67 in-frame fusion transcripts, including three recurrent fusion transcripts: FGFR3-TACCC3, RNF213-SLC26A11 and PTPRZ1-MET [139]. Fourteen other rare fusion transcripts containing sequences of genes involved in the canonical glioblastoma signalling pathways were also found.

Co-deletion of 1p/19q has been observed in up to 70% of oligodendrogliomas and 50% of mixed oligoastrocytomas [140–143]. Deletions of 1p and 19q have been associated with prolonged survival in patients with oligodendrogliomas and mixed oligoastrocytomas. Jenkins et al hypothesized that the majority of 1p and 19q deletions in gliomas were derived from a translocation t(1;19)(q10;p10), which has a prevalence of 81% in all 1p/19q deletion cases [144].

KIAA1549-BRAF translocations are characteristic of pilocytic astrocytoma [138,145]. The translocation was found by whole-genome sequencing of 96 pilocytic astrocytomas [145]. These new molecular revelations suggested that BRAF targeted therapy may be applicable in pilocytic astrocytomas since the majority of tumours harbour KIAA1549-BRAF fusions (Table 2).

Ependymoma arises from the ependymal cells of the brain and spinal cord. Surgery and irradiation are the major treatments for this disease since chemotherapy is ineffective in most patients [146]. Parker et al showed that more than two-thirds of supratentorial ependymomas contained chromosomal translocations
between avian reticuloendotheliosis viral oncogene homologue A (RELA) and chromosome 11 open reading frame 95 (C11orf95) [147]. The resulting fusion protein activates NF-κB target genes, transforming the cells [148]. This finding is significant not only for understanding the biology of the tumour but also affords potential for an effective treatment.

Conclusions

Over the past decade, FISH analysis of neoplasms has become one of the most rapidly growing areas in genomic medicine and surgical pathology practice. As more diagnostic and treatment algorithms incorporate the results of FISH, demand for the technology will become more widespread. Common FISH-detected alterations are chromosome deletions, gains, translocations, amplifications and polysomy. These chromosome alterations may have diagnostic and therapeutic implications for many tumour types. Integrating genomic testing into cancer treatment decisions poses many technical challenges, but rapid progress is being made in overcoming these precision medicine challenges. Next generation sequencing platforms allow detection of fusion genes at both the transcriptional and genomic levels. The rapidly emerging RNA sequencing (RNA-seq) technology has empowered an increased pace of fusion gene identification. RNA-seq is superior to current molecular test methods in its capacity to detect gene mutations simultaneously, the precise mapping of break-point and joint sequences, and the discovery of cryptic fusions and fusion genes with unknown partners or with short physical distance between the fusion partners.

FISH assessment of chromosomal changes is a continuously evolving technology. Its role in clarifying cancer diagnoses and its contributions to the decisions involved in choice of cancer therapy will become ever more important to surgical pathologists and the clinicians and patients they serve.

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Author contributions

LC and SZ were involved in conception and design of the paper. All the authors (LC, SZ, LW, GTM and DDD) participated in data acquisition and analysis, and writing the article. All the authors read and approved the final manuscript.

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