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Molecular pathological analysis of sarcomas using paraffin-embedded tissue: current limitations and future possibilities

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Summary

Sarcomas of soft tissue and bone are rare neoplasms that can be separated into a large number of different diagnostic entities. Over the years, a number of diagnostic markers have been developed that aid pathologists in reaching the appropriate diagnoses. Many of these markers are sarcoma-specific proteins that can be detected by immunohistochemistry in formalin-fixed, paraffin-embedded (FFPE) sections. In addition, a wide range of molecular studies have been developed that can detect gene mutations, gene amplifications or chromosomal translocations in FFPE material. Until recently, most sequencing-based approaches relied on the availability of fresh frozen tissue. However, with the advent of next-generation sequencing technologies, FFPE material is increasingly being used as a tool to identify novel immuno-histochemistry markers, gene mutations, and chromosomal translocations, and to develop diagnostic tests.

Keywords

molecular testing; next-generation sequencing; sarcoma

Introduction

Sarcomas are malignant tumours that develop from connective tissues such as muscle, fat, bone, and cartilage. A wide range of distinct sarcomas can be recognized, and many differ from each other in their clinical behaviour. The malignant tumours also need to be distinguished from the many benign lesions. Furthermore, sarcomas are rare, with an estimated number of new cases per year in the USA of 11 000 for soft tissue sarcoma and ~3000 for bone sarcomas (<http://www.seer.cancer.gov/>). Owing to the wide range of diagnostic entities and the rarity of the tumours, most pathologists do not have extensive experience in recognizing and classifying these lesions. Fortunately, the diagnosis of many tumour types is facilitated by the use of biomarkers. In most surgical pathology laboratories, the markers used consist of monoclonal antibodies that are able to detect tumour-specific antigens in formalin-fixed, paraffin-embedded (FFPE) tissue. More specialized laboratories use a range of molecular tests to aid the diagnostic surgical pathologist. These tests can

detect tumour-specific chromosomal translocations by fluorescence in-situ hybridization (FISH) or RT-PCR. Other tests identify gene-specific mutations, such as for KIT in gastrointestinal stromal tumours (GIST). The number of tumour-specific chromosomal translocations has dramatically increased over the past 15 years, and can only be expected to increase further in the future. Recently, Neuville *et al.*¹ performed a large population-based prospective study on the use of molecular testing in 1484 sarcomas. The sarcomas included GIST, well-differentiated liposarcoma and seven sarcomas associated with unique chromosomal translocations. Prior to molecular testing, the diagnoses were classified as certain, probable, or possible. It was shown that, in a variable proportion of the cases (depending on the type of sarcoma), the molecular testing contributed significantly, by either confirming a probable diagnosis or providing a diagnosis when conventional analysis was inconclusive.

It is not cost-effective and it is also labour-intensive to maintain a wide range of individual approved diagnostic tests for the many mutations and translocations that occur in the various sarcoma subtypes. The application of next-generation sequencing technology to this field opens possibilities for a more efficient and ultimately more cost-effective way of detecting these genetic abnormalities. In the past, conventional technologies for genomic studies relied on the availability of fresh frozen tissue, but next-generation sequencing can be applied to FFPE material. Therefore, our understanding of the genomic landscape of sarcomas will greatly improve with these new methods, as we can now study the wide range of entities and subtypes that are widely available as FFPE tissue. Conventional DNA sequencing required high-quality long DNA, as the goal was to sequence segments of DNA or cDNA that were as long as possible with relatively few sequencing reactions. The initial sequencing of the human genome was based on this laborious and time-consuming approach. The first complete sequencing of the human genome was started in 1990, a rough draught was reported in 2000, and the project was considered to be completed in 2003. The cost was estimated at more than 3 billion US dollars. Next-generation sequencing technology represents a dramatic shift in the approach to conventional DNA sequencing. Rather than a few reactions being performed on long fragments of DNA, millions of sequencing reactions are performed simultaneously ('massively parallel') on much shorter stretches of DNA. Powerful computer algorithms are then used to piece together the genome or the transcriptome. This next-generation sequencing approach, coupled with a dramatic decrease in the cost of these studies, has led to many discoveries in cancer biology.

The DNA or RNA isolated from FFPE tissue is fragmented, because formalin fixation and room temperature storage without special protection induces modifications and fragmentation of nucleic acids, such that only relatively short fragments of DNA and RNA strands are left that cannot be used for conventional sequencing. However, these short fragments are eminently suitable for analysis by next-generation sequencing. Thus, the maximization of data from next-generation sequencing rests on the number of simultaneous sequencing reactions that occur on unique DNA molecules (commonly referred to as 'depth'), rather than on the length of the DNA (or quality of the DNA) for Sanger DNA sequencing. The effect of formalin fixation on the nucleic acids in paraffin blocks is thought to be an ongoing process, with the result that older paraffin blocks usually yield progressively more degraded DNA and RNA. Fortunately, for clinical diagnostic purposes,

most specimens will be analysed within 6 months after paraffin embedding. A recent study by Spencer *et al.*² showed that measurable differences do exist between data obtained through next-generation sequencing from frozen tissue samples and those obtained from FFPE material. However, the differences found between FFPE material and matched frozen samples were minor, and did not affect clinical interpretation. In the same study, the investigators showed that variations in the duration of formalin fixation also did not significantly affect sequencing results. To overcome age degradation, deeper sequencing can be applied to increase the abundance (although not the percentage) of high-quality, usable reads. The protocols for extraction of nucleic acids from paraffin have improved dramatically, and the chemical modification of nucleic acids resulting from FFPE processing does not appear to interfere with next-generation sequencing reactions. Although the fragmentation of nucleic acids that occurs as a result of formalin fixation is actually a necessary step in next-generation sequencing library preparation, one major limitation in standard processing, decalcification, is worth mentioning. Tissues that are exposed to decalcification are no longer amenable to sequencing, and other studies, such as FISH and even immunohistochemistry, are also compromised; it is therefore important to save some tumour from exposure to decalcification solutions.

Molecular aspects of sarcomas

On a molecular basis, soft tissue sarcomas can be subdivided into two groups.³ In the first group, a large number of chromosomal abnormalities coexist simultaneously; these can include multiple chromosomal translocations, and gene mutations, amplifications, and deletions. The tumours in this group often show a highly aggressive histological phenotype, with significant nuclear pleomorphism and a high mitotic rate. To this group of tumours belong sarcomas such as undifferentiated pleomorphic sarcoma, pleomorphic liposarcoma, leiomyosarcoma, and others. The second group of sarcomas is characterized by having pathognomonic genetic alterations, of which three types can be discerned. The first comprises gene point mutations, such as those that occur in *KIT* or *PDGFRA* in GIST. Another genomic abnormality is that of gene amplification, and an example of this is the amplification of *MDM2* that is seen in atypical lipomatous tumour/well-differentiated liposarcoma and the dedifferentiated form of this tumour. Last, but certainly not least, are the numerous gene translocations that have been identified in soft tissue sarcomas. In a 2011 review by Demicco and Lazar, 21 soft tissue and bone tumours with diagnostic chromosomal translocations were reported.⁴ This represented a dramatic increase in the number of sarcomas with associated translocations, because, in a review in 1999, only nine sarcomas with chromosomal translocations were discussed.⁵ Since the publication of the review by Demicco and Lazar in 2011, several additional chromosomal translocations have been reported, such as the t(10;17) translocation that characterizes the novel subset of high-grade endometrial stromal sarcomas,^{6,7} the t(5;8) translocation that is found in angiofibroma,⁸ and the gene fusion between *NAB2* and *STAT6* on chromosome 12 in solitary fibrous tumours.⁹ Interestingly, chromosomal translocations have now been reported not only in malignant tumours (sarcomas), but also in benign soft tissue lesions and bone lesions. Some of these were not clearly understood to be even neoplastic. Examples are the t(17;22) translocation found in nodular fasciitis,¹⁰ the t(1;2) translocation in the lesion

formerly known as pigmented villonodular synovitis and now termed tenosynovial giant cell tumour,^{11,12} the t(16;17) translocation found in aneurysmal bone cysts,^{13,14} and the complex chromosomal rearrangements recently demonstrated in uterine leiomyomas.¹⁵

Along with the growth in the number of recognized tumour-specific chromosomal translocations, the complexity of these genetic lesions is also becoming increasingly apparent, as reviewed by Ordoñez.¹⁶ First, some sarcoma diagnostic entities can have more than one chromosomal translocation type. The most dramatic example of this is Ewing's sarcoma, in which a large number of genes can be fused with *EWSR1* (or less commonly with *FUS* and other genes) to result in the EWS phenotype. Second, a single gene can be involved in different translocations that are distinctive for multiple tumours. *EWSR1* can be found in at least 10 different tumours, including nine soft tissue sarcomas and one leukaemia, as it is joined with a range of other genes. Finally, identical chromosomal translocations can be found in diverse tumour types. A recent example of this is the finding that *YWHAE-FAM22*, which was previously reported in clear cell sarcoma of the kidney, is also present in the recently described high-grade endometrial stromal sarcoma variant.⁶

Next-generation sequencing applied to RNA derived from FFPE material

A range of molecular techniques that were initially developed on fresh frozen tissue or cell lines have been applied with variable success to DNA and/or RNA derived from FFPE tissue. Initially, genome-wide approaches relied on gene microarrays to determine either gene expression levels or gene copy number changes. Gene expression profiling with gene micro-arrays showed moderate success when cDNA derived from RNA isolated from FFPE material was used. More success was achieved with RT-PCR on FFPE material. An example of a commercial application of this approach can be found in the Oncotype test, which prognosticates breast carcinoma on the basis of the expression levels of a number of genes determined by RT-PCR on paraffin material. Array-based comparative genomic hybridization to determine gene amplification or deletion was first described by Pollack *et al.*¹⁷ using frozen tissue and subsequently paraffin material.¹⁸

Recent applications of genome-wide next-generation sequencing to FFPE material can be subdivided into several categories. The genome-wide approach should be distinguished from those techniques in which a predefined number of genes can be analysed. In contrast to these targeted applications, in which a limited number of genes are investigated, the genome-wide technologies look at essentially all DNA (whole genome sequencing) or mRNA in the specimens submitted, and thus have a broader potential for discovery. In 2010, we developed '3SEQ', a method for performing genome-wide expression profiling of mRNAs derived from FFPE tissue.¹⁹ In this technique, 3' fragments of mRNA are isolated through hybridization of the mRNA's poly-A tail to oligo-dT-coated electromagnetic beads. Next, these fragments from the 3'-ends of mRNAs are parallel-sequenced, generating short reads of between 36 bp and 100 bp. The identity of the gene from which they are derived is determined by mapping the sequencing reads to the human genome. The number of occurrences of fragments for a certain gene can then be measured, giving an accurate assessment of the level of transcription of that gene in the specimen examined. This approach does not enable sequencing of the entire length of the mRNA molecules, as one

would want to do for mutation analysis, but the technology nevertheless allows for good quantification of the number of mRNA molecules for each gene. In 2012, Lee *et al.*⁶ used next-generation sequencing on frozen tissue to identify a new chromosomal translocation defining a novel subset of endometrial stromal sarcomas. 3SEQ gene expression profiling on FFPE-derived mRNA was used to confirm the uniformity of gene expression of this new subtype of tumour, and led to the identification of a diagnostic marker for this entity.²⁰

The ability to perform 3SEQ gene expression profiling on FFPE tissue markedly increases the number of specimens and the kinds of specimen available for research. Being no longer dependent on what is available as frozen material, one can, for example, select only those cases for which clinical follow-up data are available, or analyse FFPE specimens derived from a clinical trial. In addition, as the mRNA is extracted from cores taken from FFPE blocks, very small lesions can be analysed. An example of this application can be found in the study of rare fibroblastic lesions, which are usually removed through small excisional biopsies and are often submitted in their entirety for histological analysis. For these lesions, which include giant cell tumours of the tendon sheath and others, essentially no frozen tissue is available (Figure 1). Studies such as these can be used to identify novel diagnostic markers for these fibroblastic lesions and other tumour types, but can also be used to define gene signatures for distinct subsets of fibroblastic tumour stroma that play a role in breast carcinoma behaviour.^{21–23}

In addition to enabling the study of levels of mRNA expression for conventional genes, i.e. genes that encode for cellular proteins, the 3SEQ approach can also be used to study a relatively novel class of RNA molecules called long non-coding RNA (lncRNA). The function of lncRNAs is not yet fully defined, but a major role of these molecules appears to be their ability to regulate gene transcription through their interaction with nuclear proteins.²⁴ We recently reported the genome-wide expression profile of known lncRNAs and a significant number of newly discovered candidate lncRNAs for a diverse group of tumours representing 17 different diagnostic classes from 30 carcinomas and 36 sarcomas.²⁵ The clinical relevance of this study remains to be determined, but, as lncRNAs appear to be relatively specific for different cell differentiation states, they have potential as differential diagnostic markers to distinguish soft tissue tumours derived from different cell types.

As mentioned above, there have been dramatic increases not only in the number of chromosomal translocations that have been discovered to be diagnostic for a range of tumours, but also in the complexity of these translocations. It is a major challenge for any molecular pathology laboratory to maintain approved individual diagnostic tests for each of these soft tissue and bone sarcomas. This, coupled with the rarity of the diseases, results in a very unsatisfactory cost/benefit ratio. It is probably for this reason that there are very few laboratories that offer more than the most common FISH and RT-PCR studies for diagnostic purposes. In addition, most laboratories use simple split-apart FISH tests that will not identify all possible translocations that can occur in a particular sarcoma, and that may also lack specificity if the gene tested is involved in different translocations in different tumour types. Although significant degradation does occur in FFPE material, this happens in a time-dependent fashion, and specimens that have only recently been embedded in paraffin can still yield relatively long fragments of mRNA. Given this, we hypothesized that we could

detect fusions in FFPE RNA by high-throughput sequencing. The cost of the original Illumina sequencers put this approach out of reach of most clinical laboratories. However, recently, a smaller version of the Illumina sequencer became available: MiSeq is a desktop-sized instrument that is relatively easy to operate, and its lower cost puts it within the reach of many molecular pathology laboratories. We performed next-generation sequencing on mRNA derived from FFPE tissue with a protocol that allows for long, surviving fragments of RNA in the FFPE material to be sequenced.²⁶ cDNA fragments were subjected to paired-end sequencing for 150 cycles, such that each end of a fragment results in a 150-nucleotide sequence. High-quality 50-mer sequences were identified in each fragment, and these were mapped to a custom-made library (the 'sarcomatome') that incorporates all 83 genes known to be involved in chromosomal translocations and mutations occurring in human sarcomas. Fragments where the two 50-mer sequences mapped to different genes in the sarcomatome were identified, and within this group fragments that spanned the actual breakpoint for each gene could be found (Figure 2) as further confirmation of the translocation. The 'sarcomatome' library against which the sequences obtained from the samples are matched can be easily modified to accommodate any novel translocations that may be discovered. With this approach, we could identify translocations in synovial sarcomas (three of three cases), myxoid liposarcoma (three of three cases), and clear cell sarcoma (one of one case). In two cases of Ewing's sarcoma tested, the translocation could not be identified on the MiSeq instrument, but was detectable using deeper sequencing on the original Illumina platform. Clearly, more work needs to be done to determine to what extent this approach can be used in a clinical setting on a much larger group of translocations, and also to what extent it will compare favourably or not with other approaches that use targeted high-throughput sequencing.

DNA analysis from FFPE tissue

The methods used for RNA can be applied equally to DNA. The lack of the 2' hydroxyl group means that DNA is considerably more stable than RNA; nevertheless, it does undergo extensive cleavage in archival conditions. To date, several studies have been published on the use of genome-wide high-throughput sequencing with archival DNA. Recently, we reported the results of whole genomic DNA sequencing based on FFPE tissue.²⁷ In this study of breast neoplasia progression to breast cancer, paired-end libraries were built and sequenced on the Illumina HiSeq platform, with slight variations from conventional protocols. Analysis of the mapped reads demonstrated excellent library quality that was indistinguishable from that of comparable libraries constructed from fresh DNA. This study showed the advantages of working with FFPE material, in that small samples (including ductal hyperplasias 1-3 mm in size) and samples difficult to obtain (patient-matched normal breast, hyperplasia, carcinoma *in situ*, and invasive carcinoma) could be analysed. This study and those of others²⁸⁻³⁰ show that whole genome sequencing can be performed on FFPE tissue, and it is expected that these studies will soon be extended to the study of sarcomas.

Conclusion

Traditional histology remains the most effective and cost-efficient technique for discriminating between the many different benign and malignant tumours that originate in

the soft tissues and bone. However, the diagnosis of these lesions is greatly helped by a range of immunohistochemical markers and, increasingly, by diagnostic tests that detect gene abnormalities. The advent of next-generation sequencing technology can be expected to lead to an expansion of the diagnostic tools currently available.

References

1. Neuville A, Ranchere-Vince D, Dei Tos AP, et al. Impact of molecular analysis on the final sarcoma diagnosis. *Am J Surg Pathol*. 2013; 37:1259–1268. [PubMed: 23774173]
2. Spencer DH, Sehn JK, Abel HJ, Watson MA, Pfeifer JD, Duncavage EJ. Comparison of clinical targeted next-generation sequence data from formalin-fixed and fresh-frozen tissue specimens. *J Mol Diagn*. 2013; 15:623–633. [PubMed: 23810758]
3. Nielsen TO, West RB. Translating gene expression into clinical care: sarcomas as a paradigm. *J Clin Oncol*. 2010; 28:1796–1805. [PubMed: 20194847]
4. Demicco EG, Lazar AJ. Clinicopathologic considerations: how can we fine tune our approach to sarcoma? *Semin Oncol*. 2011; 38(Suppl. 3):S3–S18. [PubMed: 22055969]
5. Fisher C. Current aspects of the pathology of soft tissue sarcomas. *Semin Radiat Oncol*. 1999; 9:315–327. [PubMed: 10516379]
6. Lee C-H, Ou WB, Marino-Enriquez A, et al. 14-3-3 fusion oncogenes in high-grade endometrial stromal sarcoma. *Proc Natl Acad Sci USA*. 2012; 109:929–934. [PubMed: 22223660]
7. Lee C-H, Marino-Enriquez A, Ou W, et al. The clinicopathologic features of YWHAE-FAM22 endometrial stromal sarcomas. *Am J Surg Pathol*. 2012; 36:641–653. [PubMed: 22456610]
8. Jin Y, Moller E, Nord KH, et al. Fusion of the AHRR and NCOA2 genes through a recurrent translocation t(5;8)(p15; q13) in soft tissue angiofibroma results in upregulation of aryl hydrocarbon receptor target genes. *Genes Chromosom Cancer*. 2012; 51:510–520. [PubMed: 22337624]
9. Chmielecki J, Crago AM, Rosenberg M, et al. Whole-exome sequencing identifies a recurrent NAB2-STAT6 fusion in solitary fibrous tumors. *Nat Genet*. 2013; 45:131–132. [PubMed: 23313954]
10. Erickson-Johnson MR, Chou MM, Evers BR, et al. Nodular fasciitis: a novel model of transient neoplasia induced by MYH9-USP6 gene fusion. *Lab Invest*. 2011; 91:1427–1433. [PubMed: 21826056]
11. West RB, Rubin BP, Miller MA, et al. A landscape effect in tenosynovial giant-cell tumor from activation of CSF1 expression by a translocation in a minority of tumor cells. *Proc Natl Acad Sci USA*. 2006; 103:690–695. [PubMed: 16407111]
12. Cupp JS, Miller MA, Montgomery KD, et al. Translocation and expression of CSF1 in pigmented villonodular synovitis, tenosynovial giant cell tumor, rheumatoid arthritis and other reactive synovitides. *Am J Surg Pathol*. 2007; 31:970–976. [PubMed: 17527089]
13. Oliveira AM, Perez-Atayde AR, Inwards CY, et al. USP6 and CDH11 oncogenes identify the neoplastic cell in primary aneurysmal bone cysts and are absent in so-called secondary aneurysmal bone cysts. *Am J Pathol*. 2004; 165:1773–1780. [PubMed: 15509545]
14. Oliveira AM, Chou MM. USP6-induced neoplasms: the biologic spectrum of aneurysmal bone cyst and nodular fasciitis. *Hum Pathol*. 2013 Epub ahead of print. 10.1016/j.humpath.2013.03.005
15. Mehine M, Kaasinen E, Makinen N, et al. Characterization of uterine leiomyomas by whole-genome sequencing. *N Engl J Med*. 2013; 369:43–53. [PubMed: 23738515]
16. Ordonez JL, Osuna D, Herrero D, de Alava E, Madoz-Gúrpide J. Advances in Ewing's sarcoma research: where are we now and what lies ahead? *Cancer Res*. 2009; 69:7140–7150. [PubMed: 19738075]
17. Pollack JR, Sørli T, Perou CM, et al. Microarray analysis reveals a major direct role of DNA copy number alteration in the transcriptional program of human breast tumors. *Proc Natl Acad Sci USA*. 2002; 99:12963–12968. [PubMed: 12297621]
18. Linn SC, West RB, Pollack JR, et al. Gene expression patterns and gene copy number changes in dermatofibrosarcoma protuberans. *Am J Pathol*. 2003; 163:2383–2395. [PubMed: 14633610]

19. Beck AH, Weng Z, Witten DM, et al. 3'-End sequencing for expression quantification (3SEQ) from archival tumor samples. *PLoS One*. 2010; 5:e8768. [PubMed: 20098735]
20. Lee C-H, Ali RH, Rouzbahman M, et al. Cyclin D1 as a diagnostic immunomarker for endometrial stromal sarcoma with YW-HAE-FAM22 rearrangement. *Am J Surg Pathol*. 2012; 36:1562–1570. [PubMed: 22982899]
21. West RB, Nuyten DSA, Subramanian S, et al. Determination of stromal signatures in breast carcinoma. *PLoS Biol*. 2005; 3:e187. [PubMed: 15869330]
22. Beck AH, Espinosa I, Gilks CB, van de Rijn M, West RB. The fibromatosis signature defines a robust stromal response in breast carcinoma. *Lab Invest*. 2008; 88:591–601. [PubMed: 18414401]
23. Beck AH, Espinosa I, Edris B, et al. The macrophage colony-stimulating factor 1 response signature in breast carcinoma. *Clin Cancer Res*. 2009; 15:778–787. [PubMed: 19188147]
24. Rinn JL, Chang HY. Genome regulation by long noncoding RNAs. *Annu Rev Biochem*. 2012; 81:145–166. [PubMed: 22663078]
25. Brunner AL, Beck AH, Edris B, et al. Transcriptional profiling of long non-coding RNAs and novel transcribed regions across a diverse panel of archived human cancers. *Genome Biol*. 2012; 13:R75. Epub ahead of print. [PubMed: 22929540]
26. Sweeney RT, Zhang B, Zhu SX, et al. Desktop transcriptome sequencing from archival tissue to identify clinically relevant translocations. *Am J Surg Pathol*. 2013; 37:796–803. [PubMed: 23598961]
27. Newburger DE, Kashef-Haghighi D, Weng Z, et al. Genome evolution during progression to breast cancer. *Genome Res*. 2013; 23:1097–1108. [PubMed: 23568837]
28. Beltran H, Yelensky R, Frampton GM, et al. Targeted next-generation sequencing of advanced prostate cancer identifies potential therapeutic targets and disease heterogeneity. *Eur Urol*. 2013; 63:920–926. [PubMed: 22981675]
29. Kerick M, Isau M, Timmermann B, et al. Targeted high throughput sequencing in clinical cancer settings: formaldehyde fixed-paraffin embedded (FFPE) tumor tissues, input amount and tumor heterogeneity. *BMC Med Genomics*. 2011; 4:68–80. [PubMed: 21958464]
30. Schweiger MR, Kerick M, Timmermann B, et al. Genome-wide massively parallel sequencing of formaldehyde fixed-paraffin embedded (FFPE) tumor tissues for copy-number- and mutation-analysis. *PLoS One*. 2009; 4:e5548. [PubMed: 19440246]

Figure 1.

Heat map of differentially expressed genes in small fibroblastic tumours obtained through 3SEQ-based gene expression profiling from formalin-fixed, paraffin-embedded material. DFSP, dermatofibrosarcoma protuberans; DTF, desmoid-type fibromatosis; NF, nodular fasciitis; NPAF, nasopharyngeal angiofibroma; PF, palmar fibromatosis; SFT, solitary fibrous tumour.

Figure 2. Schematic representation of the use of desktop transcriptome sequencing to determine the presence of chromosomal translocations in formalin-fixed, paraffin-embedded material (figure modified from Sweeney *et al.*²⁶).