

Role of NGS based RNA fusion testing in Anatomical Pathology

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Pathobiology of oncogenic fusion genes

- Gene fusion could result from reciprocal translocation, insertion, deletion, tandem duplication, inversion or chromothripsis
- Overexpression of an oncogene through promoter exchange: *TMPRSS2::ERG*
- Causing truncations which may result in loss of negative regulatory microRNA binding sites: *MYB::NFIB*
- Destroying the intrinsic control mechanism through introduction of oligomerization domains of fusion partner genes: *ALK, FGFR, NTRK, RET, ROS1*
- Loss of autoinhibitory segment, membrane, or nuclear localization domains: *BRAF*
- Impairs function by destroying the folding capacity of protein: *MLL*
- Destruction of the regulatory role of a protein: *NAB2::STAT6, PML::RARA*

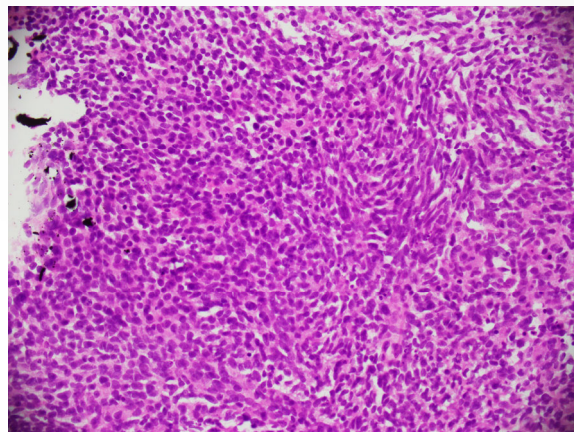


NGS based fusion testing

- Chromosome analysis, FISH and RT-PCR have been used conventionally to detect gene rearrangements
- Only one or a limited number of fusion partners can be screened by these conventional tests for each sample
- Tumours can show several fusion partners to one gene
- Hybrid capture method that uses probes that target 507 partner genes for recurrent translocation in various cancers
- Unique oligonucleotide indexes are added to each library, providing the option for downstream multiplexed sequencing



Case A

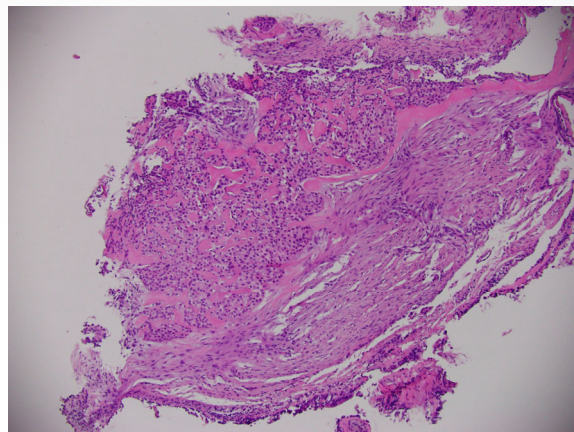


Case A: *EWSR1::WT1* fusion

- Chromosomal translocations between *EWSR1* and various genes encoding transcription factors result in the production of chimeric proteins that are involved in tumorigenesis
- The *EWSR1-WT1* fusion transcript causes activation of neural gene activation
- *EWSR1-WT1* fusion is diagnostic of desmoplastic small round cell tumour



Case B

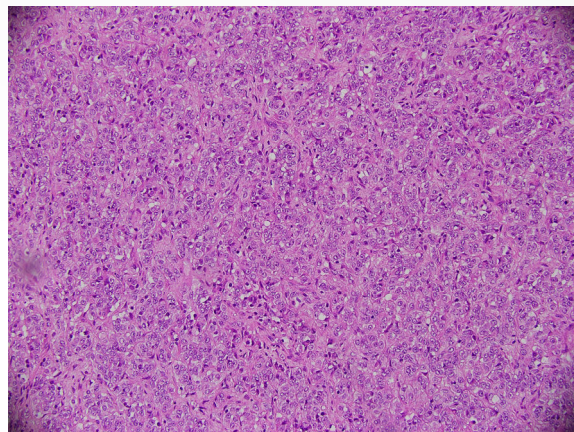


Case B: *EWSR1-ATF1* fusion transcript

- An *EWSR1-ATF1* fusion is of diagnostic value in angiomatoid fibrous histiocytoma and clear cell sarcoma of soft tissue
- In thoracic salivary gland type tumours, this fusion is seen in hyalinising clear cell carcinoma



Case C

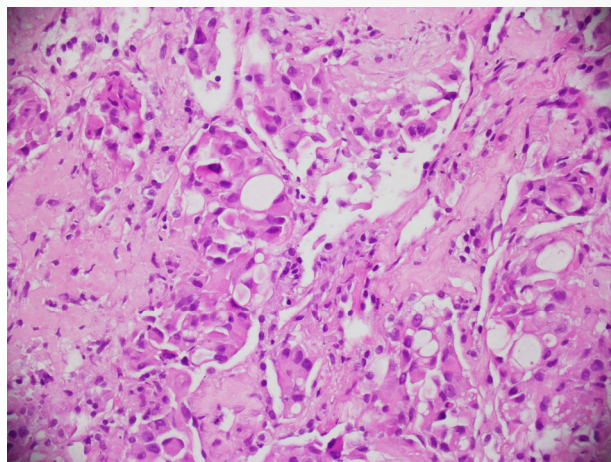


Case C: *ESR1-NCOA3* fusion transcript

- *ESR1* or *GREB1* fusions are of diagnostic significance in Uterine Tumour Resembling Ovarian Sex Cord Tumour (UTROSCT)
- Presence of *GREB1* fusion may increase likelihood of malignant behaviour
- *GREB1*-rearranged UTROSCT tend to occur in older women as compared to *ESR1*-rearranged UTROSCT



Case D

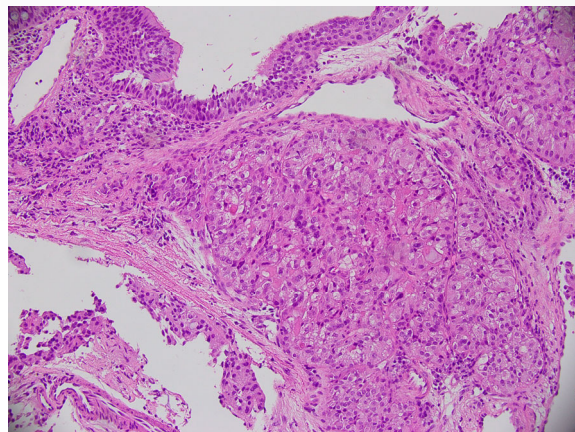


Case D: *WWTR1-CAMTA1* fusion transcript

- *WWTR1-CAMTA1* fusion is diagnostic of epithelioid hemangioendothelioma



Case E

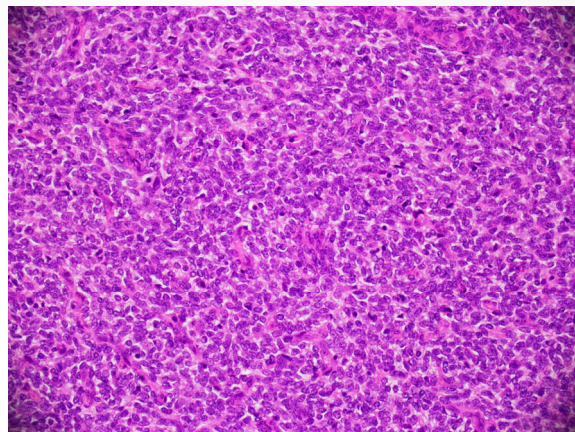


Case E: *CRTC3::MAML2* fusion transcript

- Most mucoepidermoid carcinomas (MECs) harbour a tumour type-specific translocation at t(11;19) (q21;p13) expressing *CRTC1::MAML2* fusion gene
- Rare cases display a t(11;15)(q21;q26) translocation with *CRTC3::MAML2* fusion
- Some studies indicate that fusion-positive MECs are diagnosed at an earlier stage with a lower grade and a better prognosis than fusion negative tumours



Case F

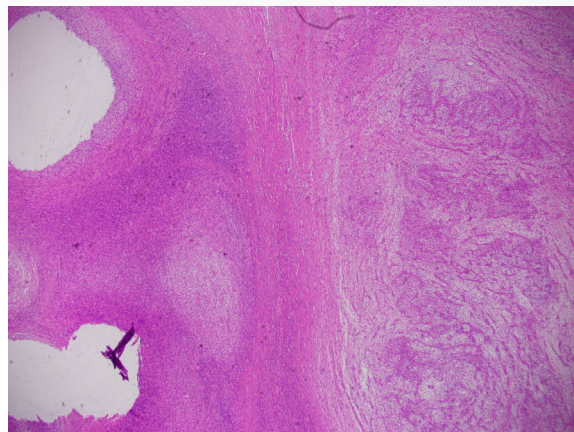


Case F: *JAZF1::SUZ12* fusion transcript

- A *JAZF1::SUZ12* fusion has been associated with endometrial stromal nodules and low-grade endometrial stromal sarcomas (LG-ESS)
- In addition, LG-ESS also harbour PHF1 and EPC1 gene fusions
- The tumours harbouring gene fusions present as earlier-stage tumors
- The high-grade endometrial stromal sarcomas harbour *YWHAE::NUTM2* or *ZC3H7B::BCOR* fusions



Case G

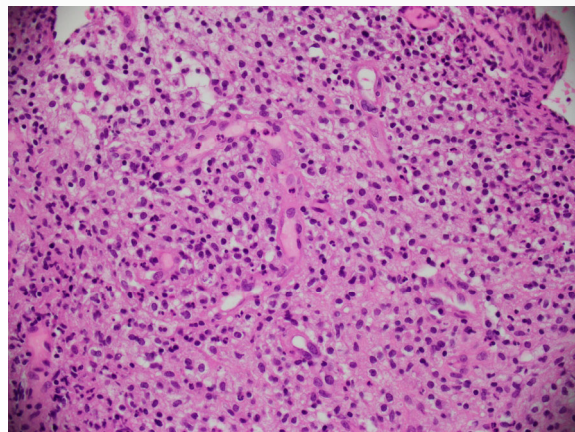


Case G: *FUS::CREB3L2* fusion transcript

- *FUS::CREB3L2* and *FUS::CREB3L1* fusion transcripts are diagnostic of Low-grade fibromyxoid sarcoma



Case H



Case H: *NPM1-ALK* fusion transcript

- This fusion gene is a frequent event in anaplastic large T-cell lymphoma
- About 80% of patients present with this fusion
- The *NPM1-ALK* oncogenic tyrosine kinase is constitutively active, triggering numerous cell signalling pathways
- Benefit from Crizotinib or Alectinib in CD-30 positive anaplastic large T-cell cutaneous lymphoma



Take Home Message

- When undertaking a molecular testing (including fusion testing) we should have specific question in mind
- Very rarely provides surprising results
- Important to understand the limitations of the assay (tissue, panel etc)
- Same fusion can be found across various tumours types, ranging from benign to malignant
- Reading molecular results in isolation can be dangerous
- Should be considered an ancillary test for anatomical pathologist, just as IHC
- Final interpretation rests with the anatomical pathologist

