Neurotrophic tropomyosin or tyrosine receptor kinase (NTRK) genes

Runjan Chetty

ABSTRACT
The neurotrophic tropomyosin or tyrosine receptor kinase (NTRK) genes (1-3) are proto-oncogenes that when activated are encountered in a wide array of tumours. The recent advent of very specific and selective inhibitors of their gene fusions makes the NTRK gene fusions actionable. NTRK gene fusions are very characteristic of specific tumours: salivary mammary analogue secretory carcinoma, breast secretory carcinoma, infantile fibrosarcoma and congenital mesoblastic nephroma. Over 90% of these tumours bear NTRK gene fusions. While next-generation sequencing is the current platform of choice for the detection of NTRK fusions, immunohistochemistry also shows great promise. Immunohistochemical localisation of the fusion protein to the nucleus, cytoplasm, nuclear membrane and cell membrane is indicative of specific gene fusions involving the NTRK genes.

The tropomyosins or tyrosine receptor kinases (TRKs) are a receptor family composed of three transmembrane proteins: TRKA, TRKB and TRKC. These proteins are in turn encoded by three neurotrophic tropomyosin/tyrosine receptor kinase (NTRK) genes: NTRK1, NTRK2 and NTRK3. Fusions of these proto-oncogenes have been encountered in a wide variety of cancers, and the importance of their detection is heightened by the recent development of very specific, selective inhibitors of NTRK gene fusions. The availability of drugs that target NTRK proteins makes the NTRK genes actionable if rearranged.

NTRK GENES
NTRK1
Is located on chromosome 1 at cytogenetic band 1q21-22 and contains 17 exons spanning 25 kb of DNA, of which exon 9 is alternatively spliced. The gene was discovered in 1982 in colon cancer. The protein produced is a membrane-bound receptor that binds with neurotrophin (NT), undergoes phosphorylation and then phosphorylates the mitogen-activated protein kinase (MAPK) pathway. The TRKA protein induces cell differentiation and may play a role in determining sensory neuron subtypes. Mutations in this gene have been associated with congenital insensitivity to pain, anhidrosis, self-mutilating behaviour, cognitive disability and cancer.

NTRK2
Is located at cytogenetic band 9q22 and consists of 24 exons. TRKB plays a role in the development and maturation of both central and the peripheral nervous systems by influencing neuron survival, proliferation, migration, differentiation, and synapse formation and plasticity. NTRK2 is involved in learning and memory, and mutations have been associated with obesity and mood disorders.

NTRK3
It is found on chromosome 15q25, and TRKC is pivotal in cell differentiation and in the development of proprioceptive neurons. TRKC receptor is found in the hippocampus, cerebral cortex and granular layer of the cerebellum.

A detailed overview of TRK in development and physiology is provided by Cocco and colleagues, and the reader is directed to this comprehensive publication.

NTRK signalling
As mentioned above, the three NTRK genes (1-3) encode for TRKA, TRKB and TRKC, respectively, and their receptors span the cell membrane with intracellular, transmembrane and extracellular domains (see figure 1). The extracellular domain is structured for specific ligand binding, and the intracellular domain has a kinase domain. The ligands bind as homodimers with NTs in the following manner: TRKA binds with nerve growth factor (NGF), TRKB with brain-derived growth factor or NT-4, and TRKC with NT3. NT3 has the potential of binding with and activating TRKA and TRKB as well but has a higher affinity for TRKC. None of the other ligands, other than NT3, have the potential to bind with more than one of the TRK receptors.

Once these specific ligand-receptor bindings occur in the extracellular domains, homodimerisation occurs followed by transactivation of the intracellular tyrosine kinase domains and recruitment of cytoplasmic adaptors. These interactions then set in motion key pathways (figure 1).

1. The NGF-TRKA binding activates the mitogen-activated protein kinase pathway which, in turn, results in cell proliferation and growth. In addition, the phospholipase Cγ (PLCγ) and phosphatidylinositol-3-kinase (PI3K) pathways are also activated.

2. The brain-derived growth factor (BDNF)-TRKB coupling activates the retrovirus-associated DNA sequences (Ras)-extracellular signal-regulated kinase, PI3K and PLCγ pathways.

3. The NT3-TRKC docking results in the activation of the PI3K-protein kinase B (PKB) pathway.
Gene of the month

Figure 1 Schematic representation of the three Trk receptors A, B and C and their respective ligands. On binding, major pathways are cascaded leading to normal functioning. Should gene fusions occur with other gene, then overexpression of NTRK proteins occur leading to an exaggeration of these normal functions. BDGF, brain-derived growth factor; ERK, extracellular signal-regulated kinase; NGF, nerve growth factor; NTRK, neurotrophic tropomyosin/tyrosine receptor kinase; PKB, phosphatidylinositol-3-kinase; PLC, phospholipase C; NTRK, neurotrophic tropomyosin/tyrosine receptor kinase. Figure 1. Schematic representation of the three Trk receptors A, B and C and their respective ligands. On binding, major pathways are cascaded leading to normal functioning. Should gene fusions occur with other gene, then overexpression of NTRK proteins occur leading to an exaggeration of these normal functions. BDGF, brain-derived growth factor; ERK, extracellular signal-regulated kinase; NGF, nerve growth factor; NTRK, neurotrophic tropomyosin/tyrosine receptor kinase; PKB, phosphatidylinositol-3-kinase; PLC, phospholipase C; NTRK, neurotrophic tropomyosin/tyrosine receptor kinase.

MECHANISMS OF NTRK ACTIVATION IN CANCER

Somatic mutations

These have been encountered in a host of malignancies: colorectal cancer, lung cancer, melanoma and acute myeloid leukaemia. The exact mechanisms by which somatic mutations in NTRK initiate and promote carcinogenesis and progression of cancers is still unknown.

Activating splice variants

Activating splice variants and in-frame deletion mutations of NTRK1 have been identified in neuroblastoma and acute myeloid leukaemia, respectively.

NTRK gene fusions

Gene fusions are the principal mechanism that unleashes the oncogenic potential of the NTRK1-3 genes. The mechanism of NTRK gene fusion is remarkably consistent: the 3' region (including the kinase domain) of the NTRK genes is fused with a 5' sequence of a fusion partner gene resulting from an intrachromosomal or interchromosomal rearrangement. This results in a chimeric oncogenic type of the kinase domain of the NTRK genes is fused with a 5' sequence of a fusion partner gene resulting from an intrachromosomal or interchromosomal rearrangement. This results in a chimeric oncogene typified by ligand-dependent constitutive activation of TRK.

Numerous cancers (breast, lung and neuroblastoma) have been shown to demonstrate TRK overexpression. Furthermore, Nakagawara et al showed that neuroblastomas that overexpressed TRKA and TRKC were associated with a better prognosis.

There are several routes by which NTRK gene fusions may be detected: next-generation sequencing (NGS), targeted RNA sequencing, reverse transcriptase PCR, fluorescent in situ hybridisation and immunohistochemistry.

With high throughput NGS being the mainstay currently deployed for NTRK fusions, several unique partner genes have been documented, often just in a single case of a particular tumour.

As can be seen in Table 1, the more common partners are ETV6, LMNA and p53.

<table>
<thead>
<tr>
<th>Genes fused</th>
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<tr>
<td>I. Tumours with &gt;90% fusions</td>
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<tr>
<td>Salivary gland mammary analogue secretory carcinoma</td>
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<td>Breast ductal carcinoma</td>
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<tr>
<td>Acute myeloid leukaemia</td>
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<tr>
<td>Acute lymphoblastic leukaemia</td>
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<td>Multiple myeloma</td>
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<tr>
<td>Cholangiocarcinoma</td>
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<td>Colorectal cancer</td>
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<td>Uterine sarcoma</td>
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<td>II. Tumours with 5%-25% fusions</td>
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<tr>
<td>Thyroid papillary carcinoma</td>
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<tr>
<td>Gastrointestinal stromal tumour</td>
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<td>Spitzoid melanoma</td>
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<td>III. Tumours with 1%-5% fusions</td>
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<tr>
<td>High-grade gliomas/glioblastoma multiforme</td>
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<td>Low-grade glioma/pilocytic astrocytoma</td>
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<td>Head and neck squamous carcinoma</td>
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<td>Lung non-small cell carcinoma</td>
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<td>Lung large cell neuroendocrine carcinoma</td>
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CANCERS/TUMOURS ASSOCIATED WITH NTRK GENE FUSIONS

The first cancers in which NTRK gene fusions were demonstrated were colorectal and papillary thyroid cancers. Subsequently, a plethora of tumours have been encountered harbouring NTRK fusions (see Table 1).

Cocco and colleagues grouped the frequency of occurrence of tumours containing these fusions into two categories: (1) rare tumours with a high frequency (>90%) of NTRK fusions and (2) common tumours with a lower frequency of gene fusions (<5% and 5%-25%) as illustrated in Table 1.

USE OF IMMUNOHISTOCHEMISTRY

NGS and targeted RNA testing for NTRK fusions is de rigueur at the moment. However, the appeal of using immunohistochemistry routinely is clear for multiple reasons: it is within the remit of most laboratories, is less expensive and labour intensive than NGSs, is quicker and allows for on-slide interpretation of localisation and patterns of staining within tumour cells.

Currently, there is a commercially available monoclonal pan-TRK antibody targeted to an amino acid sequence 880 to the C-terminus end of TRK A, B and C. Also available is an anti-TRKA monoclonal antibody that detects residues surrounding tyrosine 791 of TRKA.
Interpretation of staining

Cytoplasmic staining: it is thought that >50% of tumour cells must show cytoplasmic decoration for the case to be regarded as NTRK positive.14

Nuclear staining: any nuclear staining is taken as evidence of a positive stain.14

Several patterns of staining are encountered depending on staining localisation: cytoplasmic, nuclear, perinuclear and membrane (figure 2A–C).

The study by Rudzinski et al14 advocates that the pan-TRK antibody is better than the TRKA antibody in detecting NTRK fusions and has a sensitivity of 97%. Hechtman et al13 showed that 95% of cases with NTRK fusions stained positively with the pan-TRK antibody, and the antibody has 92% sensitivity and 100% specificity for tumours containing a NTRK fusion.

Thus, there is good evidence to support the role of immunohistochemistry as a reliable and excellent surrogate technique to detect NTRK gene fusions.

Indeed, the staining patterns obtained are also very characteristic and can be indicative of particular fusions, which in turn may help direct specific molecular interrogation.

NTRK1/2-rearranged tumours showed only cytoplasmic staining, while NTRK3-fused tumours displayed nuclear staining, with or without accompanying cytoplasmic staining.14

Hechtman and colleagues found even more specific staining patterns13: (1) tumours with TPM3/4-NTRK1/3 fusions display cellular membrane staining (figure 2A); (2) tumours with ETV6-NTRK3 and EML4-NTRK3 fusions showed nuclear staining (figure 2B); and (3) tumours with LMNA-NTRK1 fusion show perinuclear membrane staining (figure 2C).

Using similar antibodies, Chiang et al15 noted weak, diffuse TRKA and strong, diffuse panTRK cytoplasmic staining for uterine leiomyosarcomas that are NTRK fusion negative. Hung and colleagues16 also described weak staining in spindle cell tumours not harbouring NTRK rearrangements. This study showed that diffuse pan-TRK staining was present in 8% of NTRK fusion-negative spindle-cell tumours, including 50% of primitive myxoid mesenchymal tumours of infancy, 33% of fibrous hamartomas of infancy, 15% of fibrosarcomatous dermo- tofibrosarcomas protuberans, 10% of low-grade myofibroblastic sarcomas, 7% of myofibromas and 5% of spindle-cell rhabdomyosarcomas.16

Another immunohistochemical study showed pan-TRK immunoreactivity in approximately 10% of soft tissue sarcomas, with more 90% of desmoplastic small round cell tumours displaying immunoreactivity.17

The significance of staining in the absence of NTRK fusions may reflect that only NTRK gene fusions were sought and somatic mutations/activating splice variants were not looked for. Alternatively, it may reflect that the antibody is detecting normal expression of TRK protein in rare examples or a reflection of the antibodies used in the study (polyclonal vs monoclonal) and other technical considerations (fixation variation, retrieval protocols and so on).

In summary, immunohistochemistry is excellent (with high sensitivity and specificity) at confirming the presence of NTRK fusions in cases that have molecular alterations previously performed. Given the rarity of NTRK fusions in common malignancies, and the possibility that TRK immunohistochemistry may be positive in non-NTRK fused tumours, militates against its deployment as a NTRK screening tool currently.

TRK INHIBITORS

An in-depth discussion of TRK inhibitors is beyond the scope of this short review. The two inhibitors that are available are larotrectinib and entrectinib. The former is a selective inhibitor of TRKA, TRKB and TRKC and results in dramatic clinical response.8 However, as with many inhibitors, resistance occurs and second-generation TRK inhibitors are in clinical trials.

Take home messages

- There are three neurotrophic tropomyosin/tyrosine receptor kinase (NTRK) genes (1-3) encoding three proteins: TRKA, TRKB and TRKC.
- When specific ligands bind to the TRK receptors, pathways are activated that result in proliferation, survival, invasion and angiogenesis.
- NTRK fusions are found in many tumours most notably salivary mammary analogue secretory carcinoma, breast secretory carcinoma, infantile fibrosarcoma and congenital mesoblastic nephroma.
- Immunohistochemistry is a useful surrogate for NTRK fusions, and there are characteristic staining patterns for some of the fusions.
- Specific selective inhibitors to NTRK fusions are available.

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