Assessing the Prevalence of a Novel Oncogene, \textit{EML4-NTRK3}, in Archival Fibrosarcoma Samples

**Review of Literature**

**Infantile Fibrosarcoma**
- A rare soft tissue tumor, but the most common sarcoma of infancy (Hashemi et al., 2013)
- Occurs in infants less than two years of age (Obermeier et al., 1993)
- Accounts for 10% of all sarcomas in the adolescent population (Fisher et al., 1996)
- Good prognosis (Grier et al., 2006)
- 80% - 90% survival rate (Lambelle et al., 1993)
- 10% metastasis rate (Hashemi et al., 2013)
- Chromosomal translocations: a cause of malignancy (Nambiar et al., 2008)

**Chromosomal Translocation**
- The interchange of parts between two non-homologous chromosomes (Hashemi et al., 2013)
- Vary in size and complexity (Knezevich et al., 1998)
- Characteristic infantile fibrosarcoma translocation: \textit{ETV6-NTRK3} t(12;15)(p13;q25) (Knezevich et al., 1998)

**Methods**
- iRB-approved protocol (AAAO-1305): accessed 25 de-identified fibrosarcoma patient tumor specimens
- Use of 2 positive & 7 negative controls
- RNA was extracted by QIAsymphony FPPT protocol & miRNeasy FFPE kit
- Specimens were prepared for performance of RT-PCR (X5)

**Hypothesis**

\[ H_0: \text{EML4-NTRK3 is prevalent in a subset of patients diagnosed with fibrosarcoma} \]

**Research Question**

How prevalent is the EML4-NTRK3 gene in fibrosarcoma patients?

**Discussion**

- Likely that patient 2 was the primary patient
- Probable detection in patients: 4, 6, 7, 9, 10, 11, 17, 18, 23, 24
- Potential primer annealing to non-complementary sequences
- Inconsistent results → incomplete conclusions

---

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Sample type</th>
<th>Sample type</th>
<th>Sample type</th>
<th>Sample type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>Negative control</td>
<td>Negative control</td>
<td>Negative control</td>
<td>Negative control</td>
</tr>
<tr>
<td>Positive control</td>
<td>Positive control</td>
<td>Positive control</td>
<td>Positive control</td>
<td>Positive control</td>
</tr>
<tr>
<td>Negative control</td>
<td>Negative control</td>
<td>Negative control</td>
<td>Negative control</td>
<td>Negative control</td>
</tr>
<tr>
<td>Negative control</td>
<td>Negative control</td>
<td>Negative control</td>
<td>Negative control</td>
<td>Negative control</td>
</tr>
<tr>
<td>Negative control</td>
<td>Negative control</td>
<td>Negative control</td>
<td>Negative control</td>
<td>Negative control</td>
</tr>
</tbody>
</table>

**5 PCR Assay: Frequency of Detected EML4-NTRK3 Transcripts in 25 Fibrosarcoma Patient Archival Samples**
ETV6 Gene
- Located on chromosome twelve [E. de Broussolle et al., (2004)]
- A member of the ETS family: large family of transcription factors [Seth et al., 1992]
- Transcription factors: thought to influence angiogenesis and early hematopoiesis [Barrett et al., 1987; Deardorff et al., 1996]
- Some rearrangements: ETV6 fusions with genes: ABL, Trp53, and NTRK3 [Seth et al., 2000]

NTRK3 Gene
- Located on chromosome 15 (de Broussolle, 2004)
- Involved in cell growth & development of the central nervous system [de Broussolle, 1999; lecointre et al., 1998]
- Common fusion partner with ETV6 [Jouve et al., 1997]

EML4-ALK
- ALK gene- cytogenetic location 2p23 (Kwok, 2000)
- Novel to non-small cell lung cancers [Perner, 2008; Hiraoka et al., 2010]
- Potent oncogenic activity [Kwok, 2000]

Case Study
- Left distal radius lytic soft tissue mass detected on a 9 month old male infant
- Biopsy 12/7/12: diagnosed with infantile fibrosarcoma
- 1/10/13: total resection of mass
- July 2013: detection of metastases to the lungs
- Admitted to Pediatric ICU and subsequently treated with full course of chemotherapy & radiation
- Due to unusual clinical progression of the disease, patient’s DNA was sequenced
- Novel chromosomal translocation detected: t(2;15)(p21;15q25)
- Physical qPCR to encode EML4-NTRK3
- The patient achieved a complete response after 1 year of therapy
- Remains in remission

Patient Archival Samples
- 25 de-identified fibrosarcoma patient archival samples
- One of the 25 samples is known to be the primary patient
- All 5 repetitions of the assay: will detect the EML4-NTRK3 transcript in one of the 25 samples

Results

Future Research
- Repeition of experiment
- Different type of assay
- "FISH" Analysis: Fluorescence In Situ Hybridization
- Samples sent to our collaborators
- Confirmatory testing
- Specifically synthesized "probe" to detect the presence of EML4-NTRK3

Conclusions
- Metastatic or recurrent fibrosarcoma is generally deemed a terminal disease
- Study suggests: fibrosarcoma patients with EML4-NTRK3 may be cured with conventional chemotherapy & radiotherapy

Debates and Discussion
- Malignant fibrosarcoma has a poor prognosis and is typically treated with surgery, radiation, and chemotherapy
- EML4-ALK and NTRK3 fusions have been implicated in the development and progression of certain types of cancer
- The use of FISH analysis to detect EML4-NTRK3 may provide a novel therapeutic target for these patients

Bibliography