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P3.107: Hi-C Sequencing and Long-Read RNA Sequencing Identify Non-Canonical NRG1 Fusions and Splice Isoforms Informing Clinical Care

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Background and Methods

- 15-20% of lung adenocarcinoma cases (and more in squamous) are classified as “driver-negative” by short-read DNA and RNA next-generation sequencing (NGS)
- Hi-C chemistry is an NGS based method that can be performed on formalin-fixed paraffin-embedded (FFPE) tissue to identify 3-dimensional chromosome interactions. These 3-D chromosome interactions can identify fusion events, including either chimeric gene fusions predicting fusion proteins or enhancer-hijacking events in which the downstream gene is overexpressed
- Long-read RNA sequencing is a method that can be performed on fresh or frozen tissue to produce read lengths of ~ 1-10 kb, enabling identification of specific isoform expression
- We sought to identify whether Hi-C sequencing and/or long-read RNA sequencing can identify actionable genomic alterations in NSCLC**
- Hi-C sequencing was performed on 118 “driver-negative” NSCLC cases using the Arima Aventa FusionPlus test and long-read RNA sequencing was performed on 35 tumor/normal pairs of early-stage surgically resected NSCLC cases using PacBio and analyzed with FLAIR

Results

Hi-C and long-read RNA sequencing identify NRG1 fusions at a high rate

Figure 1. Of the 118 “driver-negative” NSCLC cases sequenced with Hi-C, 5 (4%) were identified to have intergenic-NRG1 fusions. Two of these fusions were identified prospectively (both adenocarcinoma). Both patients were treated with zenocotuzumab and both had clinical and radiographic response within 6 weeks.

Of the 35 tumor/normal pairs of early-stage NSCLC, 2 tumors (6%) were identified to have canonical NRG1 fusions (both adenocarcinoma).

Conclusions

Hi-C can identify clinically-actionable NRG1 rearrangements

- Hi-C sequencing was performed on 118 NSCLC cases with negative NGS
- 5 cases (4%) were found to have intergenic-NRG1 fusions
- Two of these cases were identified prospectively, with both patients responding clinically and radiographically to zenocotuzumab within 6 weeks

Long-read RNA sequencing can identify canonical NRG1 fusions and identify the specific isoforms expressed

- 2/35 NSCLC cases contained a canonical NRG1 fusion (CD74-NRG1 or SLC3A2-NRG1)
- Both fusion positive tumor samples show massive expression of NRG1 relative to their paired normal tissue
- NRG1-β is the predominant isoform expressed in these fusion cases

Future Directions

- Expand retrospective and prospective Hi-C sequencing cohorts in NSCLC and assess efficacy of targeted agents to these noncanonical fusions
- Continued analysis of long-read RNA sequencing cohort to identify novel drivers and splice isoforms that are missed by other sequencing technologies

Results

Hi-C identifies nonchimeric intergenic-NRG1 fusions and long-read RNA-seq identifies isoform-specific expression of canonical NRG1 fusions

Figure 2. A, Heatmap from Hi-C sequencing of one case of a noncanonical *NRG1* fusion showing a DNA breakpoint between an intergenic region on chromosome 8p and intron 1 of *NRG1*. Approximate locations on hg38 are shown in Mb. B, Cartoon showing proposed mechanism of these noncanonical *NRG1* fusions, with a putative enhancer region present in the intergenic region fused upstream of exon 1b. C, Schematic of *NRG1* DNA with a subset of exons annotated (transcript NM_013964.5, with the addition of exon 1 from transcript NM_001159995.3, and exon 7 from transcript NM_013956.5), a canonical chimeric CD74-NRG1 fusion, and the two noncanonical NRG1 fusions identified prospectively by Hi-C. D, NRG1 gene expression (left) and exon expression of the EGF-like domains (right) in two noncanonical NRG1 fusions identified prospectively in the Hi-C cohort, as compared with 34 control wild-type (WT) cases. E, Number of reads (left) and percent of reads (right) of NRG1 exons / isoforms that are expressed in the tumor/normal pairs of NRG1 fusion-positive cases in the long-read RNA-seq cohort.