Pan Solid Tumor Identification of NRG1 Fusions Utilizing RNA Sequencing Identifies Diverse Fusion Partners and Highlights a Lack of Co-occurring Oncogenic Driver Alterations.

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BACKGROUND

- The neuregulin 1 gene (*NRG1*) encodes an epidermal growth factor (EGF) family protein that mediates signaling via ERBB receptor pathways.[1]
- In human cancer, *NRG1* promotes cell proliferation through gene rearrangement events that preserve the EGF domain leading to constitutive activation of MAPK and PI3K signaling pathways. [2]
- *NRG1* fusions have been identified across all solid tumors at a prevalence of <1%, except invasive mucinous lung adenocarcinoma (IMA), which has a prevalence of ~10-30%. [3]
- *NRG1* fusions are key genomic drivers in patients with solid tumors that otherwise lack classical targetable alterations.[3]
- In this study, we aim to describe the landscape of *NRG1* fusions detected across solid tumors by RNA sequencing, and characterize their associations with other genomic alterations, TMB, PD-L1 status, and cell proliferation (CP) and tumor immunogenic (TIGS) signatures.

METHODS

- We analyzed 3288 FFPE patient samples from non-small cell lung (NSCLC, n=1696), breast (n=369), colorectal (CRC, n=611), esophageal (EC, n=117), ovarian (n=105), pancreatic (n=157), and unknown primary (CUP, n=233) carcinomas with comprehensive genomic profiling (CGP), including RNA sequencing for gene fusions and gene expression and as well as DNA sequencing for detection of biomarkers and genomic alterations (GAs).[4]
- OmniSeq® INSIGHT was utilized for CGP in this study and is a next generation sequencing-based (NGS) assay for the detection of genomic variants, signatures, and immune gene expression in FFPE tumor tissue. DNA sequencing with hybrid capture is used to detect small variants in the full exonic coding region of 523 genes (single and multi-nucleotide substitutions, insertions, and deletions), copy number alterations in 59 genes (gains and losses), as well as analysis of microsatellite instability (MSI) and tumor mutational burden (TMB) genomic signatures. RNA is sequenced with hybrid capture is used to detect fusions and splice variants in 55 genes, in addition to mRNA expression in 64 immune genes. Amplicon-based targeted NGS for digital gene expression (RNA-seq) was used to interrogate a panel of 395 immune genes (64 clinically validated) including T-cell receptor signaling, tumor infiltrating lymphocytes, and cancer testis antigens. Absolute reads were normalized using a non-transcript control to determine and subtract background and then compared to housekeeping genes to give a normalized reads per million (nRPM) for each gene. Expression ranks for each gene were calculated by converting nRPM values to a percentile rank between 0 and 100 as compared against a reference population of 735 solid tumor samples spanning 35 tumor types.
- A tumor immunogenic signature (TIGS) based on the mean nRPM rank of 161 immune genes was calculated to describe the degree of immune activity in each tissue sample. TIGS is considered high when ≥ 67, medium when ≥ 45 and < 67, and low when <45. A cell proliferation (CP) signature was also calculated by taking the mean nRPM for 10 cell proliferation related genes to characterize the tumor proliferation state in each tissue sample. The CP signature is considered high when ≥ 67, medium when ≥ 35 and < 67, and low when <35.

RESULTS

Patients profiled by Comprehensive Genomic Profiling

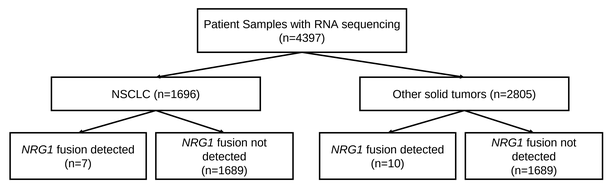

Figure 1: Number of patient samples with successful RNA sequencing separated into NSCLC and other tumor types, with the *NRG1* fusion positive cases broken out.

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RESULTS

	Non-Small Cell Lung Cancer (n=1696)	Tumor types other than NSCLC (n=2805)	All cases sequenced (n=4397)
NRG1 fusions detected, n (%)	7 (0.41%)	10 (0.35%)	17 (0.38%)
Mean age (range)	72 (64-83)	65 (41-86)	68(41-86)
Sex, n (%)			
Male	2(29%)	4(40%)	6 (35%)
Female	5(71%)	6(60%)	11 (65%)
Stage, N (%)			
III	-	1 (10%)	1 (6%)
IV	1(14%)	5 (50%)	6 (35%)
Unknown	6 (86%)	4 (40%)	10 (59%)
Specimen site, n (%)			
Primary	6 (86%)	5 (50%)	11 (65%)
Distant metastasis	1 (14%)	5 (50%)	6 (35%)

Table 1: Patient Demographics

Frequency of *NRG1* alterations by tumor type

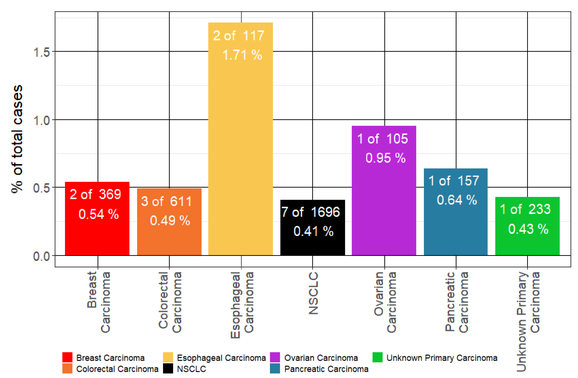


Figure 1:
Frequency of *NRG1* gene fusions across solid tumor types. Each tumor type that had *NRG1* fusions identified is listed here with the number of cases containing *NRG1* fusions and is compared to the total number of cases with RNA sequencing.

Histology of select *NRG1* fusion positive cases

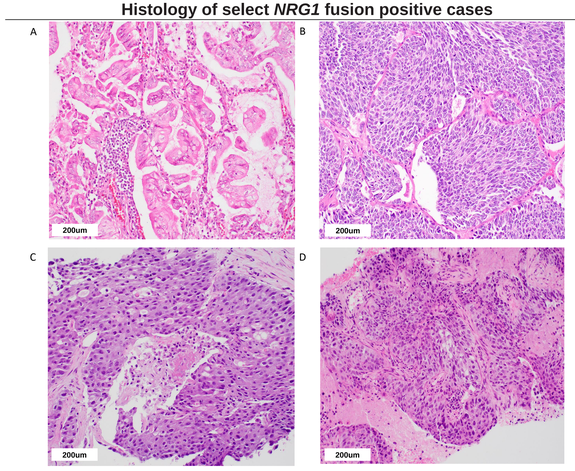


Figure 2:
NRG1 fusions are found in a wide variety of tumor types and histologies. Photomicrographs represent NSCLC samples where *NRG1* fusions were identified. A) Mucinous lung adenocarcinoma, B) Lung large cell neuroendocrine carcinoma, C) poorly differentiated lung adenocarcinoma, D) Lung squamous cell carcinoma. Images were taken at 20x and the scale bars are 200 um.

RESULTS

NRG1 Gene	Length (kbp)	Fusion Partner Gene
Intron 1 for type II, IV and V NRG1 isoforms	955 kbp	UBXN8
Intron 1 for type III NRG1 isoforms	406 kbp	DDHD2, FUT10, IKBKB, PCM1, TMEM66, ZCCHC7
Intron 2 for type III NRG1 isoforms	9.5 kbp	TNFRSF10B
Intron 3 for type III NRG1 isoforms	8.8 kbp	BIN3, BRE,CCAR2, CD9, CD74, ERO1L, KCTD9, SLC3A2
Intron 9 for type III NRG1 isoforms	2.8 kbp	PCM1

Table 2: Fusion locations, fusion partner genes, and intron sizes. Previously known fusions are colored green, novel fusions are colored red.

NRG1 has a complex gene structure, with six different promoters termed Type I through Type VI. All exons and introns were labeled with respect to the Type I promoter (NM_013956.5) [1, 26, 27]. We identified fusion breakpoint locations in introns 1, 2, 3, and 9 as well as the intron upstream to exon 1 (intron 1 for the Type II, IV, and V promoters), which collectively span 1.4 Megabases (Mb).

Chromosomal location of gene fusions

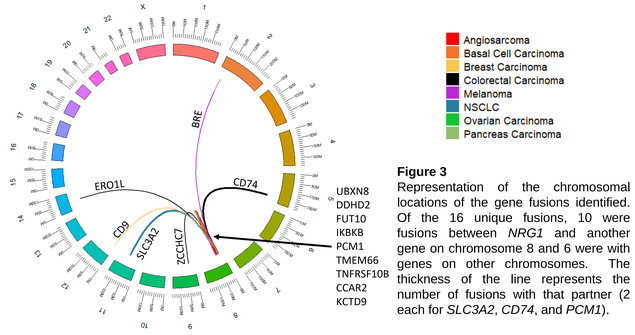


Figure 3
Representation of the chromosomal locations of the gene fusions identified. Of the 16 unique fusions, 10 were fusions between *NRG1* and another gene on chromosome 8 and 6 were with genes on other chromosomes. The thickness of the line represents the number of fusions with that partner (2 each for *SLC3A2*, *CD74*, and *PCM1*).

NRG1 gene schematic

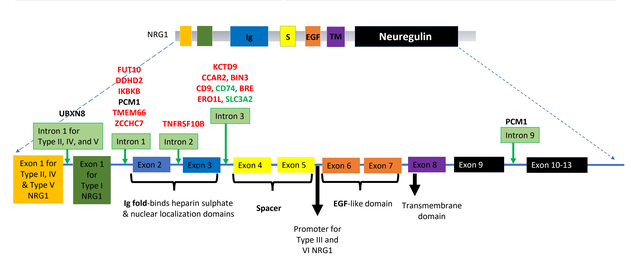


Figure 4: *NRG1* gene schematic. *NRG1* can be driven by six promoters, termed Type I through Type VI. The location of the fusion partners are listed where the fusion breakpoint occurs in the NRG1 gene. Fusion partners are color coded where red = novel partner, black = known partner identified once at that location, green = known partner identified twice. Gene schematic and exon labels are based on the reference sequence NM_013956.5.

NRG1 fusion schematics

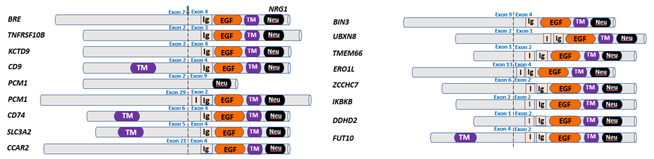
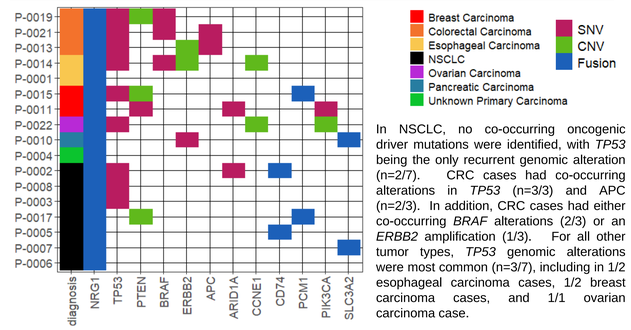


Figure 5: *NRG1* fusion schematics. All functional gene fusions have the Neuregulin (Neu) domain from the NRG1 protein.
Abbreviations: TM – Transmembrane domain. I, Ig- Immunoglobulin domain. EGF – Epidermal growth factor-like domain.

RESULTS

Co-mutation plots



In NSCLC, no co-occurring oncogenic driver mutations were identified, with *TP53* being the only recurrent genomic alteration (n=2/7). CRC cases had co-occurring alterations in *TP53* (n=3/3) and APC (n=2/3). In addition, CRC cases had either co-occurring *BRAF* alterations (2/3) or an *ERBB2* amplification (1/3). For all other tumor types, *TP53* genomic alterations were most common (n=3/7), including in 1/2 esophageal carcinoma cases, 1/2 breast carcinoma cases, and 1/1 ovarian carcinoma case.

Figure 6: Oncoprints for *NRG1* fusion cases. Only genes with an alteration in at least 2 cases are shown.

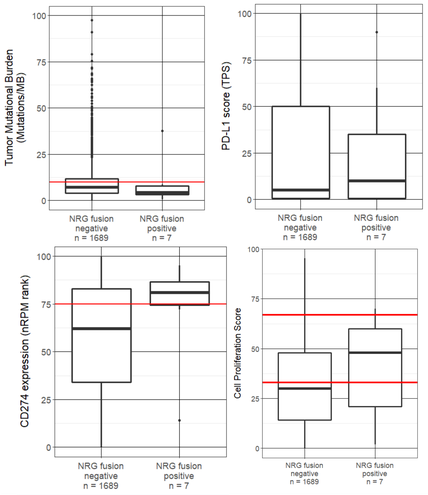


Figure 7: Comparison of Tumor Mutational Burden (A), PD-L1 TPS (B), CD274 expression (C), cell proliferation score (D) between *NRG1* fusion positive NSCLC cases and *NRG1* fusion negative NSCLC cases. The red lines in A and C represent the threshold between high and low. The red lines in D and E represent the threshold between low, intermediate, and high. Abbreviations: MB, megabase; TPS, tumor proportion score. nRPM, normalized reads per million rank. CP, cell proliferation.

CONCLUSIONS

- The array of fusion partners and large intronic areas where breakpoints occur make identification of *NRG1* gene fusions challenging.
- In this cohort, the overall incidence of *NRG1* fusions was 0.4%, twice as high as reported in a prior RNA amplicon based study (0.2%) and eight times as high as a previously reported hybrid-capture DNA based assay (0.05%). The higher detection frequency reported in our work compared to DNA based detection methodologies is likely due to fusions missed by DNA only methodologies.
- The array of fusion partners and large intronic areas where breakpoints occur make identification of *NRG1* gene fusions challenging. The potential area for rearrangements of 1.4 Mb is larger than the total size of most DNA CGP panels.
- The NSCLC *NRG1* fusion positive cases in this cohort had no co-occurring driver alterations.
- Taken together, these data highlight the importance of identifying *NRG1* fusions as these patients often lack other driver alterations and targetable biomarkers. RNA sequencing increases the detection rate for *NRG1* fusions and offers another potential therapy option for patients with advanced cancer.