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Detection of NRG1 gene fusions in solid tumors

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Abstract

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Background: *NRG1* gene fusions are rare but potentially actionable oncogenic drivers present in some solid tumors. Details regarding the incidence of these gene rearrangements are lacking. Here, we assessed the incidence of *NRG1* fusions across multiple tumor types and described fusion partners.

Methods: Tumor specimens submitted for molecular profiling at a CLIA-certified genomics laboratory and that underwent fusion testing by anchored multiplex PCR for targeted RNA sequencing were retrospectively identified. The overall and tumor-specific incidence was noted as was the specific fusion partner.

Results: Out of 21,858 tumor specimens profiled from September 2015 to December 2018, 41 cases (0.2%) harbored an *NRG1* fusion. Multiple fusion partners were identified. Fusion events were seen across tumor types. The greatest incidence was in non-small cell lung cancer (25), though this represented only 0.3% of non-small cell lung cancer cases tested. Other tumor types harboring an *NRG1* fusion included gallbladder cancer, renal cell carcinoma, bladder cancer, ovarian cancer, pancreatic cancer, breast cancer, neuroendocrine tumor, sarcoma, and colorectal cancer.

Conclusion: *NRG1* fusions can be detected at a low incidence across multiple tumor types with significant heterogeneity in fusion partner.

Keywords

NRG1; Neuregulin-1; gene fusions; NSCLC; RNA-sequencing

Introduction:

Appropriate management of advanced non-small cell lung cancer (NSCLC) is guided by the presence or absence of specific molecular drivers. The identification of activating genomic alterations in *EGFR*, *ALK*, *ROS1* or *BRAF* not only provides insight into the underlying biology but also directs initial and subsequent therapeutic decisions (1–5). It is now standard of care to search for these mutations and fusions in all patients with non-squamous NSCLC (6). It has also become clear that some molecular drivers will serve as therapeutic targets across multiple tumor types (7), including the tumor agnostic approval of larotrectinib for tumors with a gene fusion in *NTRK1*, *NTRK2*, or *NTRK3 (8)*. As our understanding of cancer grows increasingly sophisticated, additional drivers have surfaced that may have a similar impact on evolving treatment paradigms.

Neuregulin-1 (*NRG1*) gene fusions are an emerging, potentially actionable oncogenic driver (9). *NRG1* fusions can promote pathologic signaling via MAPK and other canonical pathways (10). When *NRG1* fusions are present, targeting ERBB2 and ERBB3 has been an effective treatment strategy *in vitro.* Recently, clinical responses to tyrosine kinase inhibitors and monoclonal antibodies have also been reported (9,11–13).

The interest in evaluating the prevalence of *NRG1* fusions has increased given the potential therapeutic implications of this genetic alteration. Since the original description of the *CD74-NRG1* gene fusion in invasive mucinous lung adenocarcinoma, detection has been noted in other tumor types, both *de novo* and as a resistance mechanism in *ALK* rearranged

NSCLC (9,14–16). Here, we report the incidence and characteristics of *NRG1* fusions across a variety of tumor types based on a large molecular profiling experience.

Methods:

Patient Cohort

An institutional review board (IRB)-approved, retrospective assessment of a de-identified molecular profiling database was surveyed for solid tumors that underwent fusion testing. From a cohort including all cases submitted to a CLIA-certified laboratory (Caris Life Sciences, Phoenix, AZ) for comprehensive genomic profiling from September 2015 to December 2018, all unique cases that underwent successful fusion testing for targeted RNA sequencing were identified. Additionally, all histologic characteristics were reviewed by a board-certified pathologist (Z.G.).

Gene Fusion Detection

Prior to any molecular analysis, H&E-stained sections of formalin-fixed paraffin-embedded (FFPE) tumor tissue were manually assessed by board-certified pathologists for tumor cell populations and harvested using manual microdissection to enrich the sample to at least 20% tumor nuclei. Anchored multiplex PCR was performed for targeted RNA sequencing using the ArcherDx fusion assay (Archer FusionPlex Solid Tumor panel). RNA sequencing was performed on mRNA that was isolated and reverse transcribed into complementary DNA from FFPE tumor tissues. Unidirectional gene-specific primers were used to enrich for target regions, followed by Next-Generation sequencing (Illumina MiSeq platform). Targets included 52 genes, and the full list can be found at http://archerdx.com/fusionplex assays/solid-tumor (accessed 12/27/18). Reads that were matched to a database of known fusions and other oncogenic isoforms (Quiver database, ArcherDx), as well as those novel isoforms or fusions with high reads (>10% of total reads) and high confidence after bioinformatic filtering, were analyzed. Samples with less than 4,000 unique RNA reads were reported as indeterminate and excluded from analysis. All *NRG1* transcript variants were investigated whereby splice junctions were analyzed using the UCSC genome browser to predict the likelihood of the mRNA transcript to encode a functional protein (17). The detection sensitivity of the assay allows for detection of a fusion that is present in at least 10% of the cells in the samples tested.

Frame Retention Prediction

NRG1 fusions were predicted to be (1) in-frame variants, (2) out of frame variants of unknown significance or (3) translated variants where exon 2 of *NRG1* is spliced to upstream non-coding exons with confirmed presence of internal initiation sites, (e.g. methionine codon) (17,18). Inclusion of these variants was based on the retention of the EGF-like domain of *NRG1*, the functional domain which facilitates its oncogenic potential (19).

Next Generation Sequencing

Next-Generation Sequencing (NGS) was performed on isolated genomic DNA using the Illumina NextSeq platform. A custom-designed SureSelect XT assay was used to enrich 592

whole-gene targets (Agilent Technologies, Santa Clara, CA). All variants were detected with > 99% confidence based on allele frequency and amplicon coverage, with an average sequencing depth of coverage of > 500 and an analytic sensitivity of 5%. For variant classification, variants of genes that were pre-determined for their cancer-related and clinical significance were interpreted by board-certified molecular geneticists and categorized as pathogenic, presumed pathogenic, variant of unknown significance, presumed benign, or benign according to ACMG (American College of Medical Genetics and Genomics) standards. Only pathogenic or presumed pathogenic mutations were considered deleterious and included for assessment of co-mutation patterns with *NRG1*-fusion positive cases.

Immunohistochemistry (IHC)

IHC was performed using commercially available detection kits and automated staining techniques (Benchmark XT, Ventana, Tucson, AZ; and AutostainerLink 48, Dako, Carpinteria, CA). Primary antibodies tested: Her2/neu (4B5, Ventana), pan-TRK (C17F1, Cell Signaling) and ALK (D5F3, Ventana). Cutoffs for positive staining: (3) pan-TRK, ≥1+ and ≥1% of cells, (4) Her2, ≥3+ and ≥ 10% and (5) ALK, ≥3+ and ≥ 10%.

Results:

Sample population

From September 2015 to December 2018, a total of 21,858 tumor specimens from unique patients were successfully evaluated. The tumor types included NSCLC (n=9592), glioma (n=1997), colorectal cancer (n=1690), breast cancer (n=1106), bladder cancer (n=945), ovarian cancer (n=686), sarcoma (n=627), pancreatic adenocarcinoma (n=623), gallbladder cancer (n=580), other gynecological malignancies (e.g. uterine, cervical, vulvar; n=524), melanoma (n=360), prostate cancer (n=261), gastric adenocarcinoma (n=239), head and neck squamous cell carcinoma (n=236), thyroid cancer (n=219), renal cell carcinoma (n=211), neuroendocrine tumors (n=203), esophageal cancer (n=202), small cell lung cancer (n=107), extrahepatic bile duct cancer (n=98), small bowel cancer (n=98), gastrointestinal stromal tumor (n=83), hepatocellular carcinoma (n=83), thymic cancer (n=31), testicular cancer (n=25), and other malignancies (n=1032).

Incidence

The incidence of *NRG1* fusions in the entire tested population was 0.2% (41/21,858). Incidence varied by tumor type (Figure 1): 0.5% gallbladder cancer (3/580), 0.5% pancreatic cancer (3/623), 0.5% renal cell carcinoma (1/211), 0.4% ovarian cancer (3/686), 0.3% non-small cell lung cancer (25/9592), 0.2% breast cancer (2/1106), 0.2% sarcoma (1/627), 0.1% bladder cancer (1/945) and 0.1% colorectal cancer (1/1690). The remaining identified *NRG1* fusion was in a patient with a neuroendocrine tumor of the nasopharynx. Table 1 describes the characteristics of the 41 patients found to have an *NRG1* fusion. The most common histologic subtype was adenocarcinoma (70%), of which 24% were classified as mucinous adenocarcinoma and another 8% had a mixed histology with a mucinous component (Figure S1). The majority of cases were stage IV at the time of fusion detection. *NRG1* fusion events were more frequently identified in females (66%) versus males, and most specimens were procured from the primary site (68%) compared to a distant metastasis (32%). In the total

cohort, 51% (11228/21858) of patients were females and 59% (12798/21858) of specimens profiled were from primary sites.

Fusion partners

The specific fusion partners were also diverse within and across malignancies (Table S1; Figures 2–5, Figure S2). In NSCLC, *CD74* was the most common fusion partner (n=12), but other detected partners in NSCLC cases included *SDC4* (n=3), *SLC3A2* (n=1), *TNC* (n=1), *MDK* (n=1), *ATP1B1* (n=1), *DIP2B* (n=1), *RBPMS* (n=1), *MRPL13* (n=1), *ROCK1* (n=1), *DPYSL2* (n=1), and *PARP8* (n=1). In the other malignancies, the identified fusion partners were as follows: *SETD4*, *TSHZ2* and *ZMYM2* in ovarian cancer; *ADAM9* and *COX10-AS1* in breast cancer; *ATP1B1*, *CDH1* and *VTCN1* in pancreatic cancer; *NOTCH2* and *ATP1B1* (n=2) in gall bladder cancer; *POMK* in colorectal cancer; *RBPMS* in renal cell carcinoma; GDF15 in urothelial bladder cancer and *WHSC1L1* in sarcoma and *HMBOX1* in neuroendocrine tumor of the nasopharynx. Of the 41 *NRG1* fusions identified, 34 were in-frame, 3 were out of frame variants of unknown significance, and 4 were translated variants.

Co-occurrence with other genetic aberrations

NRG1 fusions were mutually exclusive with oncogenic alterations in *EGFR*, *KRAS*, *ALK*, *ROS1*, and *RET* (Figure 2). One case co-occurred with a *BRAF* G466A mutation, one with a *KRAS* G12D mutation and three with *NF1* or *NF2* mutations (*NF1*,Q616fs, NSCLC and c.204+1G>T, ovarian; *NF2* H242fs, NSCLC). Most cases (n=30) also demonstrated concurrent mutations in tumor suppressor genes, including *TP53*.

Survival

Limited survival analysis is shown in Figure S3 for patients with full annotation (n=7). Median survival for the entire cohort was 638 days and varied by tumor type, though analysis is limited by the small sample size.

Discussion:

NRG1 gene fusions represent a novel oncogenic driver across cancer types. These rare genomic events can generate proteins that retain the extracellular EGF-like domain of *NRG1* and the transmembrane domain of the specific fusion partner. These proteins then serve as ligands for ERBB3 (HER3) and ERBB4 (HER4) receptors (10). ERBB3 can then be activated through juxtacrine signaling from the EGF-like domain and autocrine signaling of secreted *NRG1* (19). Subsequent heterodimerization of ERBB3 with ERBB2 activates downstream signaling important in tumorigenesis mediated by pathways including ERK, PI3K, AKT and NFkB, described in cell models (9,19).

In this report, we retrospectively analyzed over 21,000 specimens after RNA sequencing using the ArcherDx platform to detect *NRG1* fusions. As previously reported, our study confirmed the occurrence of *NRG1* fusions in NSCLC, breast cancer, cholangiocarcinoma, ovarian cancer, and pancreatic cancer with a low overall incidence. Here, we also detected *NRG1* fusions in colorectal cancer, sarcoma, and a neuroendocrine tumor of the nasopharynx, which had not been previously reported. In this report, the majority of these

tumors (70%) were adenocarcinoma. *NRG1* fusions in NSCLC had been described more frequently in the invasive mucinous adenocarcinoma subtype; in this series, 32% (8/25) of the *NRG1*+ NSCLC cases had a mucinous histology or a mucinous component (Table 1). The use of broad molecular profiling in this series was based on clinician discretion and may be influenced by patient and tumor characteristics. Thus, the actual incidence may not be entirely representative of the general population. Despite these shortcomings, the detection of such rare genetic alterations across different tumor types supports broader use of next-generation sequencing.

The specific *NRG1* fusion partners are variable within and across tumor types (11–15,20). Several novel fusion partners detected in this report include *TNC*, *MRPL13*, *MDK* and *DIP2B* in NSCLC. Previous reports suggest fusion partners may influence localization of *NRG1* to the plasma membrane (20), though the exact significance remains unclear, and the variety of partners observed may introduce challenges for widespread detection efforts.

Splice junctions of all candidate *NRG1* fusions were analyzed to predict likelihood to encode functional proteins. Most were predicted to be in-frame; however, a recurrent novel fusion class was identified whereby exon 2 of *NRG1* is spliced to upstream non-coding exons of fusion partner genes. In each of these cases (n=4; Table S1), a codon encoding for methionine is present a short distance into exon 2 of *NRG1* that could potentially act as a translation initiation codon; if functional, the fusion partner could be providing the promoter for a likely N-terminal truncated version of *NRG1*. These observations are consistent with similar studies where *NRG1* fusion variants included chimeric proteins and cases where expression of *NRG1* is controlled by the promoter of the 5’ partner (21). Alternative methodologies are needed to confirm expression of the transcript s identified in this study to determine their significance.

Additional studies describing *NRG1* fusions suggest these events are mutually exclusive with other known molecular drivers (14). This was consistent with the findings in this report. Specifically, all NSCLC cases were ALK, ROS, RET fusion-negative and KRAS wild type, and all pancreatic adenocarcinomas were KRAS wild type. The exception was one colorectal cancer case that also harbored a *KRAS* G12D mutation. The remainder of the cases studied harbored several pathogenic variants in tumor suppressor genes including *TP53* and DNA damage and response genes (*CHEK2*, *BRCA2*, *WRN*).

NRG1 fusions are detected in a variety of tumor types. In 2014, Fernandez-Cuesta and colleagues first described the *CD74-NRG1* gene fusion in five female never-smokers whose tumors lacked known activating mutations (10). As comprehensive molecular profiling and RNA sequencing has become more prevalent, *NRG1* fusions have been detected in a variety of other tumor types, including breast, ovarian, and pancreatic cancer (14,15). Analysis of MSK-IMPACT dataset including next-generation sequencing and the MSK solid fusion assay identified ten patients with *NRG1* fusions (out of 17,485 tested): seven in lung adenocarcinoma, two in pancreatic cancer, and one in breast cancer. Further analysis with RNA sequencing revealed additional fusions in other tumor types including ovarian cancer, uterine carcinosarcoma, renal clear cell carcinoma, prostate cancer, and head and neck cancer (9).

As *NRG1* alterations activate the ERBB2/ERBB3 signaling pathway, targeted treatment with inhibitors of this pathway is an appealing therapeutic strategy. Dual targeting of ERBB2 and ERBB3 has also been evaluated in preclinical models (22,23). Afatinib, a pan-ERBB inhibitor, was successfully utilized in this manner, and several patients with tumor harboring an *NRG1* fusion achieved durable benefit with afatinib (11–13). Response to an ERBB3 monoclonal antibody, GSR2849330, has also been reported (9). Combining an ERBB3 monoclonal antibody and an EGFR tyrosine kinase inhibitor was also effective in a small case series (24). Prospective studies are needed to define the role of targeted therapy for patients with tumors harboring *NRG1* fusions, but these data suggest that *NRG1* fusions represent a novel potential target across many tumor types that warrant further study.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Significance:

NRG1 fusions are potentially actionable genomic events seen in various tumor types. While there are reports of therapeutic efficacy with agents that target Erb-B2/Erb-B3, little is known about the characteristics of these fusions. Here, we report the incidence of *NRG1* fusions in a large cohort of solid tumors that underwent RNA sequencing. *NRG1* fusions were detected at a low incidence across many solid tumor types. Multiple fusion partners were identified, which will influence the development of strategies to detect these events on a large scale.

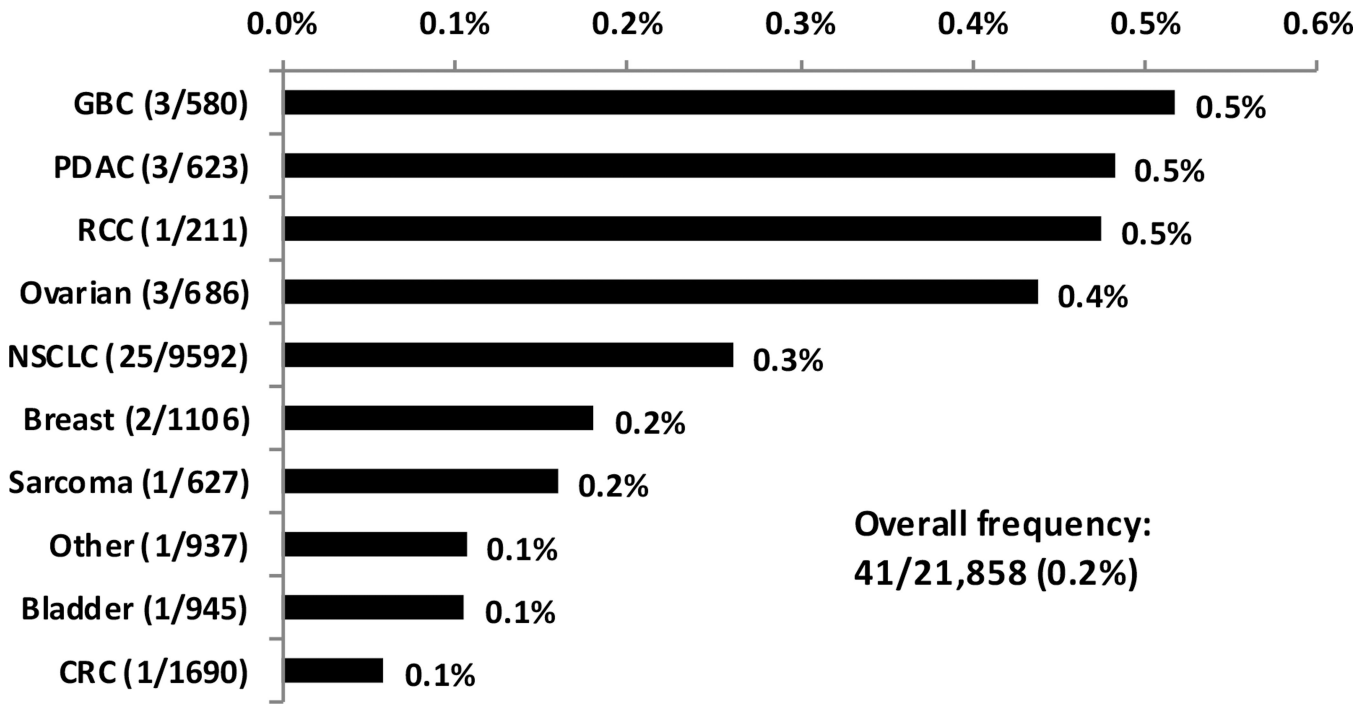

Figure 1.

Rate of *NRG1* fusions by tumor type. The tumor type with *NRG1* fusion in the other category is a neuroendocrine tumor of the nasopharynx. Abbreviations: GBC, gallbladder cancer (cholangiocarcinoma); PDAC, pancreatic ductal adenocarcinoma; RCC, renal cell carcinoma; NSCLC, non-small cell lung cancer; CRC, colorectal cancer.

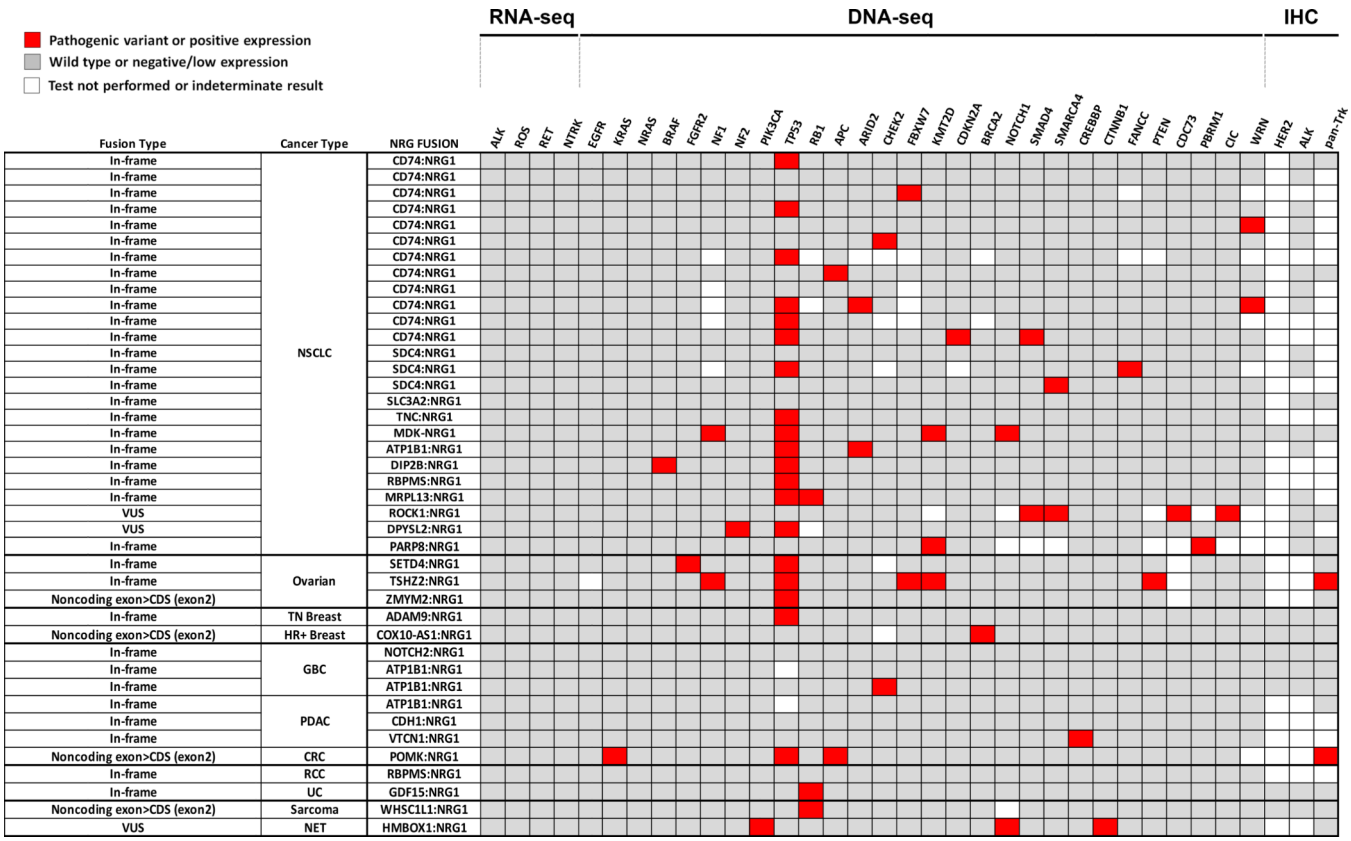

Figure 2.

Genomic features observed in *NRG1*-fusion positive solid tumors. Oncoprint plot illustrating co-occurrence of driver events, genes with any pathogenic variant detected in the cohort and other clinically relevant protein markers. Each *NRG1*-fusion positive sample corresponds to one row in the table: frame prediction of the fusion, cancer type and fusion partner are provided. Fill of boxes correlate with gene/protein status: (1) red, pathogenic variant detected or positive expression, (2) grey, wild type or low/negative expression and (3) white, test was not performed, or indeterminate. Pathogenic variants in oncogenes were rare, but at least one mutation in tumor suppressor genes including TP53 occurred in all but nine samples. Abbreviations: NSCLC, non-small cell lung cancer, TN (triple-negative) breast, HR+ (hormone receptor positive) breast, GBC, gallbladder cancer (cholangiocarcinoma); PDAC, pancreatic ductal adenocarcinoma; CRC, colorectal cancer; RCC, renal cell carcinoma; UC (urothelial bladder cancer); NET (neuroendocrine tumor of the nasopharynx); VUS, fusion variant of unknown significance; CDS, coding sequence.

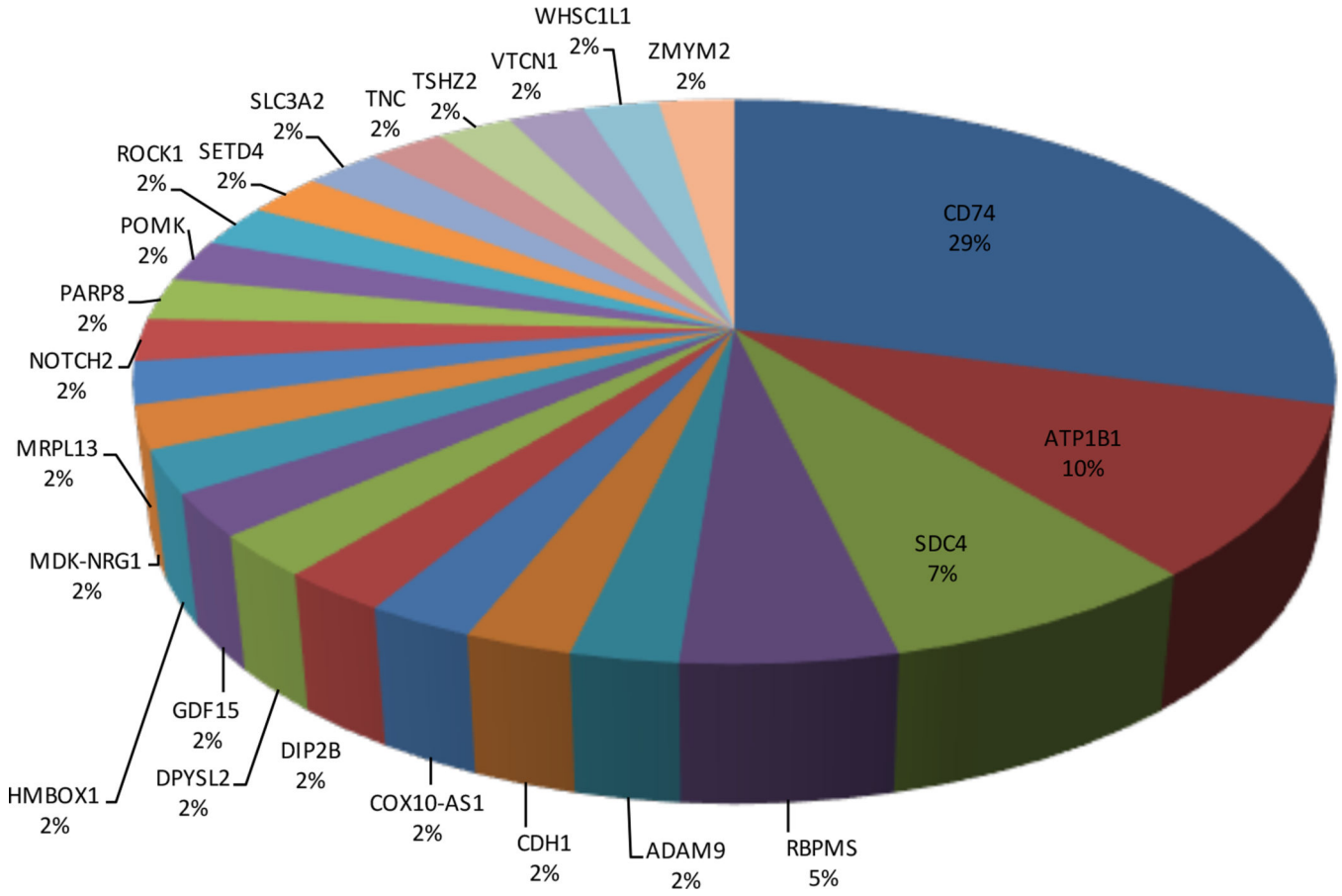

Figure 3.

NRG1 fusion partners. Pie chart showing the proportion and variety of fusion partners for *NRG1*.

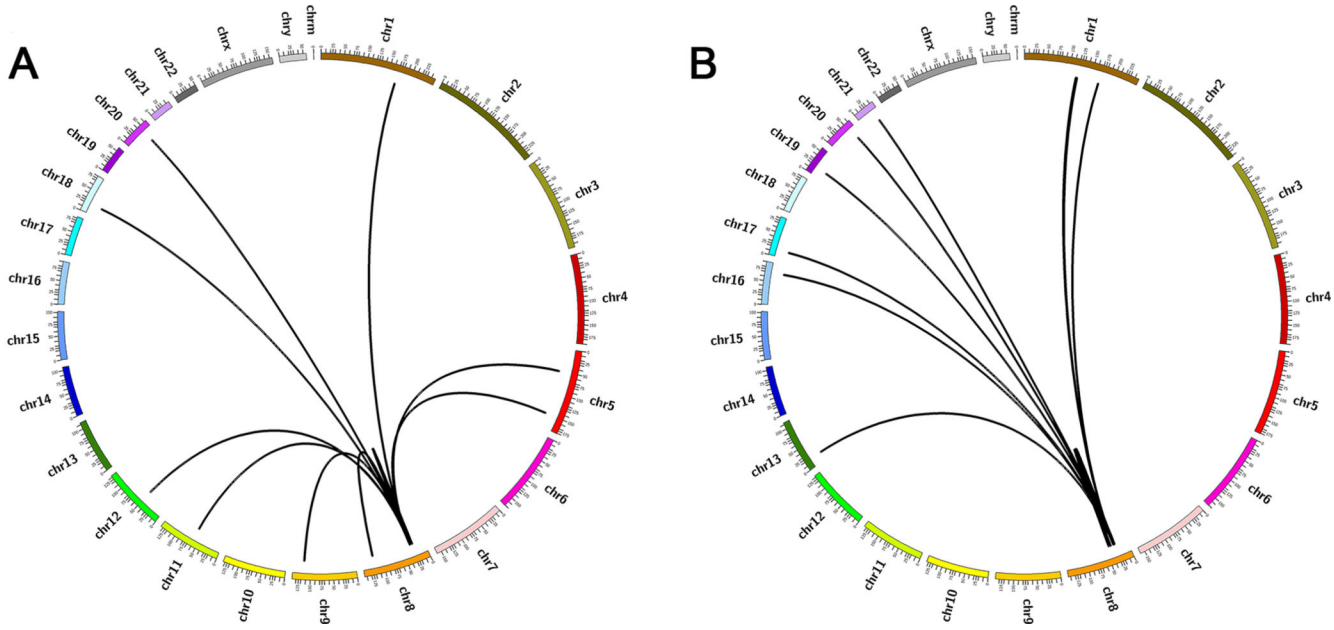

Figure 4.

Circos plot of depicting *NRG1* fusion genes and partners from Table S1. *NRG1* and partners in (A) NSCLC and (B) all other tumors

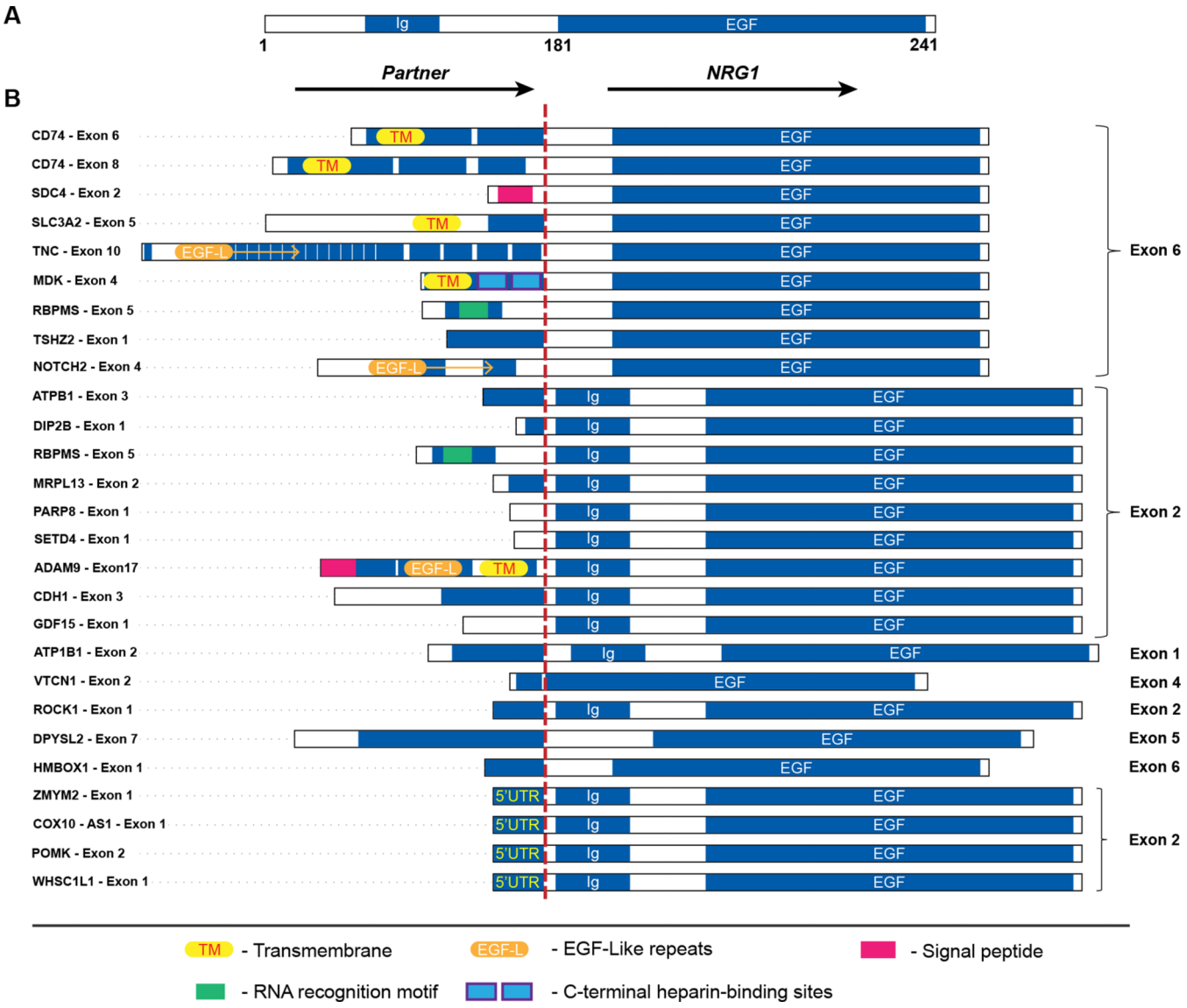

Figure 5.

Schematic diagram of *NRG1* fusion variants in solid tumors. A. Genomic structure of wild type *NRG1*; B. Fusion variants identified with 5' partners joined to 3' *NRG1*. Bars depict the predicted functional domains (not shown to scale) of interest and red dashed line indicates fusion breakpoints. The EGF domain is preserved in all fusion variants.

Table 1.

Patient and Tumor Characteristics for *NRG1* Fusion Positive Cases.

	NSCLC		Ovarian Cancer		Breast Cancer		GI Cancers[a]		GU Cancers[b]		Other[c]		Overall	
Total, n	25		3		2		7		2		2		41	
Median Age, range	71	52–90	53	47–69	44	38–49	46	37–68	61	58–63	59	36–81	68	36–90
Sex, n, %														
Male	8	32%	-	-	-	-	3	43%	1	50%	2	100%	14	34%
Female	17	68%	3	100%	2	100%	4	57%	1	50%	-	-	27	66%
Group Stage, time of biopsy, n, %	**22**		**3**		**2**		**7**		**2**		**-**		**36**	
I	1	5%	-	-	-	-	-	-	-	-	-	-	1	3%
II	3	14%	-	-	-	-	-	-	-	-	-	-	3	8%
III	6	27%	-	-	-	-	-	-	-	-	-	-	6	17%
IV	12	54%	3	100%	2	100%	7	100%	2	100%	-	-	26	72%
Histology, n, %														
Adenocarcinoma,													29	70%
Papillary	1	4%	-	-	-	-	1	14%	-	-	-	-	-	-
Mucinous	6	24%	-	-	-	-	-	-	-	-	-	-	-	-
Acinar	2	8%	-	-	-	-	-	-	-	-	-	-	-	-
Mixed[d]	3	12%	-	-	-	-	-	-	-	-	-	-	-	-
Poorly differentiated	2	8%	-	-	1	50%	-	-	1	50%	-	-	-	-
NOS[e]	8	32%	-	-	1	50%	6	86%	-	-	-	-	-	-
Squamous cell carcinoma	2	8%	-	-	-	-	-	-	-	-	-	-	2	50%
Serous carcinoma	-	-	2	67%	-	-	-	-	-	-	-	-	2	5%
Other[f]	1	4%	1	33%	-	-	-	-	1	50%	2	100%	5	20%
Specimen Site, n, %														
Primary	20	80%	3	100%	-	-	3	43%	1	50%	1	50%	28	68%
Distant Metastasis	5	20%	-	-	2	100%	4	57%	1	50%	1	50%	13	32%

a: pancreatic ductal adenocarcinoma; gallbladder cancer (cholangiocarcinoma) and colorectal cancer; GU, genitourinary

b: renal cell carcinoma, urothelial bladder cancer

c: soft tissue sarcoma of the extremity/trunk, neuroendocrine tumor of the nasopharynx

d: mixed histology (two patients with acinar, clear cell and mucinous components and one patient with adenosquamous features)

e: NOS, not otherwise specified

[f]Other, pleomorphic carcinoma or sarcoma, carcinosarcoma, clear cell carcinoma or neuroendocrine tumor. Abbreviations: NSCLC, non-small cell lung cancer; GI, gastrointestinal.